Expression of the Haemoglobin S gene on the island of Curacao

Zanen, George Eduard van

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G. E. VAN ZANEN

EXPRESSION OF THE HAEMOGLOBIN S GENE ON THE ISLAND OF CURAÇAO

STELLINGEN

1. Onder de negroide bevolking van Curaçao komen ten minste twee vormen van beta-thalassaemie voor.

2. Bij patienten met recidiverende infecties en een verlaagd serum gamma-globuline gehalte (hypogammaglobulinaemie) is immuno electrophoretisch onderzoek der serum gamma-globulinen noodzakelijk om een zgn. dysgammaglobulinaemie uit te sluiten.

3. Bij acromegalie is de uitscheiding van hydroxyproline verhoogd.

4. Eosinophiele longinfiltraten als gevolg van worminfecties berusten op de aanwezigheid van de parasiet in het longweefsel.

5. De opvatting, dat in Nederland trichocephalus dispar niet pathogeen zou zijn, is onjuist.

6. Indien bij een zuigeling met klinische en röntgenologische verschijnselen van decompensatio cordis een normale hartgrootte wordt gevonden, dan is het waarschijnlijk dat alle longvenen onder het diaphragma draineren.

7. De navraag naar de aard der mictie vormt een essentieel onderdeel van de anamnese bij de zuigelingen contrôle.

8. Bij de fractuurbehandeling is de toediening van calcium-praeparaten niet alleen overbodig, doch zelfs schadelijk.

9. De operatieve behandeling van de asymptomatische cystocèle moet als een kunstfout worden beschouwd.

10. Bij de behandeling van otitis externa diffusa dient het gebruik van vaseline als zalfbasis te worden afgeraden.

11. Tropenkolder, ook wel keerkringziekte genoemd, wordt voornamelijk in het moederland waargenomen.

12. De toelating van het medisch hoger onderwijs op de Nederlandsche Antillen impliceert de stichting van een medische universiteitsbibliotheek.

RIJKSUNIVERSITEIT TE GRONINGEN

Expression of the Haemoglobin S gene on the island of Curaçao

with a summary in Spanish

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE GRONINGEN OP GEZAG VAN DE RECTOR MAGNIFICUS DR. F. H. L. VAN OS, HOOGLERAAR IN DE FACULTEIT DER WISKUNDE EN NATUURWETENSCHAPPEN, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP MAANDAG 9 JULI 1962 DES NAMIDDAGS TE 4 UUR

DOOR

GEORGE EDUARD VAN ZANEN

GEBOREN TE SHANGHAI

DRUKKERIJ J. RUYSENDAAL - AMSTERDAM

PROMOTOR: PROF. DR. J. H. P. JONXIS

Aan mijn Ouders

Aan *Evelyn*
Maarten
Tjeerd
Steven

VOORWOORD

Bij deze studie werd medewerking ondervonden in Nederland, in Amerika en op Curaçao.

Hooggeleerde Jonxis, hooggeachte promotor, het was een voorrecht in 1958 in Uw laboratorium te werken. Onder Uw leiding heb ik deze studie kunnen voltooien, waarbij Uw critische adviezen mij ten zeerste hebben gestimuleerd. Ik betuig U hiervoor mijn hartelijke dank.

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Zeergeleerde de Rook, door onze samenwerking op Nieuw Guinea, welke voor mij van grote betekenis is geweest, wist ik welke waarde ik moest hechten aan Uw advies op Curaçao de haemoglobinopathieën nader te bestuderen.

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Jegens allen, die op enigerlei wijze hebben bijgedragen tot het tot stand komen van dit proefschrift spreek ik mijn erkentelijkheid uit.

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"It is obvious that human beings are not to be crossed like cattle or flies. But while controlled matings are impossible in human societies, we now realize that man in his time and numbers has contrived to enter into many of the matings desired by the geneticist; it remains only to locate these matings for study".

— James V. Neel —

INTRODUCTION

Population research on haemoglobinopathies is generally limited to the determination of the nature and the incidence of abnormal haemoglobins and of normal haemogoblins in different concentrations. These aberrant forms of human haemoglobins are unequally distributed over the globe, and seem to be accumulated in tropical and sub-tropical regions.

Since it was shown in 1949 that a positive result of a sickle cell test depends upon the presence of sickle cell haemoglobin in human erythrocytes, it has been found that this first abnormal haemoglobin to be discovered occurs among the present inhabitants of the Caribbean islands.

Negroids of the islands and the border regions of the Caribbean basin are partly descendants of negro slaves brought in from different regions of Africa from the 16th to the latter part of the 18th centuries. Although some correlation between the islands and the African lands of origin was still possible at first, the subsequent mingling of the races and also intermarriage between negroids from the different islands makes it impossible, except in a few rare instances, to discern the original population of the different islands. Historical data further underline that intensive intermarriage took place with races and population groups which arrived later. Thus the distribution over these islands of the gene determining S haemoglobin can be explained on historical grounds.

The occurrence of S haemoglobin in this area has been proved since 1949 by means of new laboratory methods.

As elsewhere, the serious clinical syndromes connected with the presence of this haemoglobin provided the main reason behind the investigation.

An exception among the contributions originating from the Netherlands Antilles was made by a population investigation on Curaçao, the results of which were put forward by Jonxis in 1957 during the "Symposium on Abnormal Haemoglobins", as part of a comparative study in confirmation of the malaria hypothesis.[36] This investigation established the nature and the incidence of abnormal haemoglobins on this malaria-free island, special attention being paid to the S haemoglobin owing to the purpose of the study. More data concerning the incidence

of this haemoglobin on Curaçao were given in 1959 by Van der Sar in a study dealing mainly with the clinical aspects of S and C haemoglobins.[84]

Larger-scale investigations in the Caribbean area have been rare. In this connection the desirability of further examination of the incidence and mode of inheritance of haemoglobin S among the heterogeneous population of Curaçao became apparent.

Multi-family research, the basis of modern human genetics, often encounters insurmountable difficulties in tropical regions owing to local ways of life, extra- or non-marital relations, shortage or absence of reliable records, lack of sufficient families and the difficulty of meeting the technical demands of laboratory methods under primitive circumstances, while as a rule the shortness of the periods covered constitutes a serious handicap.

These problems do not arise in Shell's medical department on the island of Curaçao, and multi-family research by means of haemoglobin electrophoresis was accordingly possible.

This department of the largest industry on the island provides free medical care for all workers and their legal families, besides taking all prophylactic and occupational health measures necessary.

The opportunity to carry out a genetic investigation during 1959-1962 was used as:

1. data of an extensive polyclinical and clinical documentation were available from the medical department, together with reliable family records from the administration department;

2. the investigation could be carried out among workers and their families, all negroids*, for the greater part born on Curaçao, and also among workers originating from the Leeward and Windward Islands, the British Caribbean islands and Surinam.

The presence of workers and their families from the last-mentioned regions who settled on Curaçao following the establishment of the oil industry in 1918, enabled the extension of the Curaçao family investigation and at the same time provided the opportunity of gaining a limited impression of the incidence of certain haemoglobin genes on the small Caribbean islands and in Surinam. This made it possible, as far as the present generation is concerned, to trace to the country of origin genes newly brought to Curaçao.

The starting point of the investigation was a non-clinical approach to

* The expression "negroid" is used in the sense given by Eibergen in his recent study i.e. an individual showing more or less clearly the external features of the negro race.[16]

sickle cell haemoglobin, as the results were available from many years' application of Emmel's sickle cell test at periodical medical examinations. For screening purposes the method proved to be reliable, within the limits also valid for other sickle cell tests. After paper-electrophoretic identification of the haemoglobin phenotype in sickle cell positive blood samples, it was possible:

1. to calculate the percentages of "Hb S positive" Curaçao workers and Curaçao married women compared with the total number of workers and married women of Curaçao ancestry examined by the sickle cell test method;

2. to establish by means of electrophoresis a pattern of S haemoglobin combinations with other haemoglobins among these Hb S positive men and women;

3. to carry out an electrophoretic haemoglobin analysis of families if one or both of the parents, regardless of the country of origin, was shown to be a haemoglobin S carrier.

Owing to the choice of the human material the results of the investigation among the Curaçao workers and their wives cannot be accepted as being representative for the population of this island. However, the determination of the nature and incidence of S haemoglobin among these groups can be considered as a complement to the afore-mentioned investigations, the more so as a totally different approach was applied to the persons born in Curaçao and not involved in the previous investigation. The Curaçao origin of the people concerned in this respect was determined by their Curaçao name and birth registration, all speculation regarding the ancestors of the adults being avoided.

This study concerns other haemoglobins only in so far as they were encountered together with haemoglobin S among the selected workers and women or were found in their partners and offspring.

The long duration for tropical circumstances of the observation (18 years maximum) must be mentioned as a peculiar advantage of the opportunity provided to carry out this investigation. Further, owing to selection by periodical medical examination, it was possible to trace S haemoglobin combinations unconnected or barely connected with pathology. The fact that in general large families were examined, together with the conspicuously low infant mortality rate on this tropical malaria-free island, was also favourable for the compilation of data.

The expression of the haemoglobin S gene on the island of Curaçao, the object of this study, comprises:

1. the mode of the incidence of haemoglobin S among men selected through labour relations, and their families, all either born on Curaçao or originating from one of the other Caribbean regions;
2. an investigation into the haemoglobin distribution in sibships;
3. the study of the biochemical and genetic relationships in certain families;
4. an investigation of the influence of haemoglobin S on the fitness of the carriers.

The aim of this study is to contribute to the knowledge of haemoglobinopathies in the Caribbean area.

PART I

LITERATURE

CHAPTER I

HAEMOGLOBIN S ON CURAÇAO.

The first literature on sickle cell anaemia and sickle cell trait on Curaçao arose from the clinical interest in the serious symptoms of sickle cell anaemia, and consequently concern the clinic and pathology. The data added concerning the incidence of the sickle cell trait among the population of this island are of little value at the present moment. Not so much because they were obtained by means of the sickle cell test, which was the only method applied and of limited value despite the specific nature of the sickle cell phenomenon, but as the population investigation was assigned a secondary part only. As a result of this insufficient attention was paid to selection and to the exact description of the persons as a group.

In 1943 Van der Sar reported the occurrence of the sickle cell trait as 8.3 % of 848 persons, for the greater part school children.[84] In 1949 the same author gave an average of 10.7 % (men 9.4 %, women 12.3 %) of 2499 persons examined, for the greater part hospital patients.[83]

At the same time Beekman of Shell Curaçao's medical department, whose "Clinical and haematological studies on sickle cell disease" represent the first thesis on this subject for Curaçao [6], had found 206 positive sickle cell tests during periodical medical examinations among 4746 negroid workers, all originating from Curaçao, the other islands of the Caribbean and Surinam. No differentiation according to the island of origin was carried out and therefore calculation of the average percentage to obtain population data is of limited value.

Finally, in 1954 a communication about "Investigations on the abnormal haemoglobin in sicklaemia and sickle cell trait" by means of electrophoresis, alkali denaturation, amino acid analysis and measurements of the ultra violet spectrum of haemoglobin originating from Curaçao mentioned the presence of this trait among 11.7% of the population.[30]

On the other hand data about the frequency and mode of the incidence of Hb S on Curaçao obtained from planned population investigation are of definite importance (Jonxis[36], and Van der Sar[84]). Here the material was well described and modern laboratory methods were applied for the haemoglobin analysis. The aim of Jonxis' study was to prove the relation between malaria and the incidence of Hb S by comparative population investigation on the malaria-free island of Curaçao and in (previously) malaria-rich Surinam. In this connection it was important that the haemoglobin material from the western part of Curaçao, the "third district", should also be considered separately, since there had been little, if any, immigration into this district owing to the employment available with the oil industry in other parts of the island. Consequently the present inhabitants are more direct descendants of the original negroes than the other negroids of the island. The low Hb S percentage of the district was of considerable importance, whilst the percentage of Hb C

carriers among this group was a strong indication that the original negroes on Curaçao came from Ghana.

The investigation involved 790 "pure negroes", including 349 from the "third district". After haemoglobin examination by electrophoresis, chromatography and alkali denaturation in the Groningen paediatric laboratory, the following percentages and haemoglobin characterizations were established:

Among all examined (790):

8.6 % AS – 7.2 % AC – 0.9 % S – 0.4 % SC – 0.4 % AF

Among those examined from the third district (349):

5.2 % AS – 8.0 % AC – 0.3 % S – 0.9 % SC – 0.9 % AF

The average age of the Hb S homozygotes examined was 19 years as against an average of 34 years for persons with normal haemoglobin; this included persons of 18, 20, 29 and 53 years of age.

Jonxis, under reservations arising from the order of the material, made a comparision of the percentage of Hb S homozygotes with the percentage to be expected if the life expectancies of these carriers were unaffected: 0.20 : 0.30 (third district material). The average age of the Hb SC carriers hardly differed from those of normal haemoglobin carriers. No cases of Hb C only were found. It is moreover important that for three persons, in the absence of abnormal haemoglobins, the presence of more than 10 % foetal haemoglobin was established.

Van der Sar's published data were obtained from a population investigation on a larger scale and are the results of a haemoglobin examination and blood group investigation among 1502 negroids, including school children, civil servants, working women, nurses, visitors to the mass-radiography health centre, and aged persons. The haemoglobin examination was carried out in the Groningen paediatric laboratory and included paper-electrophoresis, alkali denaturation and a solubility test.

The following percentages and haemoglobin characterizations were obtained:

1. among all persons examined (1502):

   6.5 % AS – 5.8 % AC – 0.3 % S – 0.4 % SC

   id from the third district (336):

   5.4 % AS – 8.0 % AC – 0.3 % S – 0.9 % SC

2. among all men examined (681):

   8.7 % AS – 5.4 % AC

among all women examined (821):

5.3 % AS – 7.4 % AC

Here no break-down by age group was given.

3. by comparing the percentages among persons with more or less pigment (defined as "dark skinned" and "light brown skinned"):

among the dark skinned from the third district (211):

5.7 % AS – 9.0 % AC – 0 % S – 0.5 % SC

among the light brown skinned from the third district (125):

4.8 % AS – 6.4 % AC – 0.8 % S – 1.6 % SC

among the dark skinned from the rest of the island (570):

9.3 % AS – 6.8 % AC – 0.7 % – 0.2 % SC

among the light brown skinned from the rest of the island (596):

4.5 % AS – 3.5 % AC – 0 % S – 0.3 % SC

4. by division into age groups:

	0-10 yrs (97) %	11-20 yrs (378) %	21-30 yrs (254) %	31-40 yrs (208) %	41-60 yrs (289) %	61-100 yrs (276) %
AS	8.3	6.4	5.9	6.7	6.9	6.1
S	2.0	0.5	0.4	0.0	0.3	0.0
SC	0.0	0.3	0.4	1.4	0.3	0.0
AC	6.2	6.4	6.3	6.3	4.3	5.4

These results showed that:

ad 1) the percentages obtained of Hb S and Hb C trait carriers among all persons examined from the whole island were lower than Jonxis' investigation had shown, while the corresponding percentages of persons from the third district only (from material of the same order) agreed quite well in both studies;

ad 2) considering the percentages of Hb S and Hb C trait carriers among all men examined on the one hand and all women examined on the other, a considerable difference becomes apparent. This difference cannot be explained, probably because no separate classification of men and women according to age groups was given;

ad 3) with regard to the persons with more or less pigment it was confirmed that Caucasian admixture involves a decrease of the per-

centage of Hb S and Hb C trait carriers whereas the relation between the traits remains the same;

ad 4) the percentage of Hb S trait carriers is highest in the first ten years of life and then decreases to remain practically constant in later decennia. This decrease of the Hb S trait percentage from 8.3 to 6.1 could not be explained. The number of children examined was low in comparison with the number of persons in the other age groups; the constant percentage of Hb C trait carriers among the persons of the four lower age groups, however, is an argument against the influence of the material choice.

Hb S homozygotes were found up to the higher age group. The calculated average age of these persons was 20 years as against an average of 36 years for persons with normal haemoglobin and with Hb S and Hb C trait, and an average of 33 years for the Hb SC heterozygotes. The incidence of the Hb S trait, calculated from AS and SC carriers, amounted to 7.0 % so the incidence of the Hb S gene was 3.5 %. The expected incidence (p^2) of the Hb S homozygotes could be calculated as $p^2 = (\frac{0.07}{2})^2 = 0.0012$, resulting in an expected proportion of Hb S heterozygotes of 0.0012 : 0.69 = about 1 : 55. In fact, however, this proportion was 5 : 104 = about 1 : 21.

Van der Sar explained this number of Hb S homozygotes, more than double that expected, by the heterogeneity of the population and by the fact that on Curaçao, owing to group and class forming, there was no question of intermarriage at random. Also the material of Van der Sar's investigation did not yield any cases of Hb C homozygosity, but six persons with normal adult haemoglobin once again showed increased percentages of foetal haemoglobin (from 1.5 to 24.0 %).

The blood group investigation (see publication of Nijenhuis[60]) confirmed that the original Curaçao negro population was brought from the Gold Coast and adjacent regions.

While the lowest percentage (about 12) of Caucasian admixture was proved among the persons of the third district, the blood group division at the same time showed that "the mixing of the races has resulted in a persisting heterogeneity, as the result of the formation of classes arising, for example, from preference for marriage partners of similar skin colour".[60]

CHAPTER II

MULTI-FAMILY RESEARCH ON HAEMOGLOBIN S AMONG NEGROIDS.

INTRODUCTION.

Haemoglobin S is the most thoroughly studied of the abnormal haemoglobins[5].

Among the staggering amount of examinations many family investigations have been performed, mostly to confirm by genetic analysis the clinical diagnosis concerning one member of the family. New laboratory methods have revealed much more regarding the phenotypic expression of the Hb S gene, but in some cases family investigations may still be essential to determine the genotype.

As the discovery of one "critical" family only may suffice to elucidate indistinct genetic relationships, incidental investigation of families in this respect has its value too. However, isolated investigation of families cannot be satisfactory for the study of genetic relationships and the expressions of genes in general as the quantity of results will be small, and these will often be obtained from separate areas involving the possibility of different racial origin.

Multi-family research on Hb S has been limited, and is characterized by its connection with the incidence of sickle cell anaemia.

The first investigations were carried out to prove Neel's hypothesis of 1947 against the views of Taliaferro and Huck which prevailed until 1949.[50, 51]

These attributed all expressions of the sickle cell phenomenon to the presence of a single dominating gene resulting in sickle cell trait, and in some people (inexplicably) in sickle cell anaemia.

In 1949 Neel was able to confirm his hypothesis that sickle cell trait and sickle cell anaemia were expressions of heterozygosity and homozygosity respectively, by the results of an investigation in which parents of sickle cell anaemia patients were examined for the presence of sickle cells.[51] In the same year Beet reached the same conclusion independently during an investigation of a large family in Africa.[7]

The importance of the haemoglobin S discovery by Pauling, Itano, Singer and Wells is that the presence of more than one kind of adult haemoglobin in humans was established. Moreover, the results of their electrophorectic investigation strongly supported these genetic assumptions, as it was proved that sickle cell trait carriers possess normal and abnormal haemoglobin, while sickle cell anaemia patients have abnormal haemoglobin but lack the normal.[61] It could therefore be concluded that

the appearance of the sickle cell phenomenon is determined by the presence of a gene; when this gene is single (heterozygosity) the phenomenon is attended by the appearance of the sickle cell trait, and when it is double (homozygosity) — each gene originating from one of the parents — sickle cell anaemia results.

This mode of inheritance of the gene determining the appearance of the sickle cell phenomenon, can be described as "semi-dominant or incompletely recessive"[59] or as a form of "intermediate inheritance", in which the "heterozygote is intermediate in appearance between the two homozygotes" and "one gene produces an abnormality, while the presence of two in the same individual produces a greater degree of abnormality".[17]

This inheritance relationship implies:

- that both parents of a homozygous child must be heterozygous;
- that a marriage between a heterozygote and a "normal" partner will produce 50 % heterozygotes and 50 % "normal" children;
- that a marriage between two heterozygotes will produce, besides "abnormal" and "normal" homozygotes, also heterozygous children in a proportion 1 : 1 : 2;
- that of a marriage between an "abnormal" and a "normal" homozygote all children will be heterozygous.

Multi-family research.

In 1951 Neel published his findings to furnish detailed data on the results of genetic studies designed to test the homozygous-heterozygous hypothesis.[52] This study, involving 465 people distributed among 75 kindreds, all negroes from south-eastern Michigan, covered sickle cell carriers and sickle cell anaemia patients known from hospital data. Several sickle cell tests were prepared by different techniques from each patient concerned, and the diagnosis of the sickle cell disease was subjected to such severe criteria that misjudgement was practically excluded.

In 1953 Neel published supplementary data on the occurrence of the sickle cell trait among the parents, having added 47 more to the 75 kindreds already mentioned.[55]

Another study, also published in 1951 and supplying genetic evidence supporting the homozygote-heterozygote hypothesis by means of the results of a multi-family investigation, came from the Belgian Congo.[43] J. and C. Lambotte–Legrand define their investigation an "étude génétique et hématologique basée sur 88 cas", and give as some of the reasons for the investigation "la grande fréquence de la maladie, sa méconnaissance près que générale, l'absence jusqu'à ce jour de toute information concernant les différentes formes de l'affection au Congo Belge". Their results were obtained from an investigation of infants and school children in Léopoldville. Blood of these native children was examined by the sickle

cell test according to Emmel. The family investigation always started from a child found to be a sickle cell carrier.

Family members of sickle cell anaemia patients were involved in a second American study by Banks, Scott and Simmons published in 1952 "because of the conflicting opinions".[4] Sickle cell slides were prepared from each person concerned according to three different techniques. Sixteen negroid families were examined.

In 1954 Vandepitte produced the results of a second multi-family investigation in the Belgian Congo.[81] Family members of hospital patients, for the greater part babies and children but also adults, were examined by means of the sickle cell slide prepared according to two techniques. This investigation covered 243 negro families in which 261 children suffered from sickle cell anaemia.

In 1959 a third publication from the Belgian Congo, by Delannay, gave inter alia the results of an electrophoretic haemoglobin examination of 65 families of workers of the "Union Minière de Panda".[12] This family investigation was started from known sickle cell anaemia patients (52 families) on the one hand, and from sickle cell trait carriers who had been traced while examining blood donors (13 families) on the other.

A great deal of the genetic material of these investigations was approached clinically. If, following Vandepitte[81] and Neel,[56] for this reason the "abnormal" homozygotes are ignored and only the segregation for sickle cell trait vs. non-sickle cell trait among the non-anaemic siblings of children with sickle cell anaemia is considered, the total results appear to confirm Neel's hypothesis (expected ratio 2 : 1).

	number S traits	number non S traits	total exam.	method
Neel ('51)	62	36	98	S test
Lambotte–Legrand ('51)	45	25	70	„
Banks et al. ('52)	15	18	33	„
Vandepitte ('54)	141	67	208	„
Delannay ('59)	95	42	137	electr.
total	358	188	546	

Significantly deviating results were found in the scanty material of Banks, Scott and Simmons who did, however, state that not all the children could be examined, while those examined were only given one sickle-cell test.[4]

On the other hand exceptions were established. Although most of the

parents of sickle cell anaemia patients were sickle cell trait carriers, non-sickling parents were found:

Neel ('51): Upon examination of both parents of 49 families of which one or more of the children were sickle cell carriers, seven parents were found not to be sickle cell carriers (one mother and six fathers; of the latter one could be excluded from paternity).

Lambotte-Legrand ('51): Starting from 88 children, all sickle cell carriers, both parents of 73 children proved to be sickle cell trait carriers. Of the parents of the remaining 15 children, three mothers and seven fathers proved to be "negative", while one mother and five fathers could not be examined. At the same time it was found that the parents in eight out of 205 families of which at least one child was a sickle cell carrier without signs of the disease, did not show sickle cells in their blood.

Banks ('52): One out of 14 mothers examined of children with the typical symptoms of sickle cell anaemia did not show the sickle cell phenomenon.

Vandepitte ('54): 231 out of 233 mothers examined proved to be sickle cell trait carriers; the same goes for 190 out of 206 fathers examined. Repeated examination of the two non-sickling mothers did not yield a positive test. Of the 16 non-sickling fathers ten were excluded from paternity by means of serological reactions.

Since heredity relationships can only be accepted fully if the exceptions to the rule can also be explained, Neel himself put forward the following genetic considerations by way of criticism:[53, 54, 55]

Discrepancy between legal and biological parenthood. Serological reactions can exclude but cannot definitely confirm parenthood, consequently a negative sickle cell test on the part of the mother is of greater significance than a corresponding result for the father.

Mutation. This, "certainly one of the most basic biological phenomena"[59] has to be considered if the union of a sickle cell positive and a sickle cell negative parent produces a child with sickle cell anaemia. The findings for sickle cell negative parents of families where only one child has sickle cell anaemia or the sickle cell trait are particularly important for the study of mutation. If several children in one family show these signs, germinal mutation appears improbable.

Since no haematological or electrophoretic abnormalities were present, mutation was first assumed in the sickle cell negative mothers in Neel's ('53) and Vandepitte's ('54) material. Owing to insufficient data this was not the case with the negative mothers from Lambotte-Legrand's material ('51). This assumption was afterwards abandoned as a result of findings which will be dealt with later on.

Vandepitte et al. calculated that mutation alone could not be the explanation of the high sickle cell trait in the Belgian Congo.[82]

According to Rucknagel and Neel no clear examples have yet been obtained of mutation resulting in the S gene.[68]

Incomplete or non-penetrance of the sickle cell gene. As genes find expression in an environment that is also confined by other genes, suppression of the usual gene effect is possible. Partial suppression could possibly be the explanation of the findings of Singer and Fisher, who by means of electrophoresis proved about 5% Hb S in a woman whose blood did not show the sickle cell phenomenon but who had children with sickle cell anaemia.[72]

Interaction of one single Hb S gene with unknown environmental or

genetic factors, or otherwise stated, sickle cell disease is assumed to have different genetic backgrounds.

During the investigations already mentioned indications were found that this could be the case.

Besides typical sickle cell anaemia,[4] the sick children of non-sickling parents also showed syndromes which were described as "an unusually mild case of the disease" or as "between sickle cell trait and sickle cell anaemia" and "unusually well compensated",[52] and ones where "le diagnostic semble le moins assuré".[43]

At the same time haematological abnormalities (including target cells, ovalocytes and microcytes) were established for some of the non-sickling parents, and also in other family members, which pointed in the same direction.[4, 52] However, with others this was not the case.[81]

Following multi-family research two of the interaction possibilities of the Hb S gene were established for the first time among negroids.

This was possible by applying electrophoresis as a new technique for haemoglobin analysis in addition to the haematological methods.

Electrophoretic investigation of some exceptional families from Neel's material showed that the typical sickle cell patients possessed two abnormal haemoglobins instead of one.[41] The second abnormal haemoglobin, to be distinguished from normal haemoglobin as well as from Hb S by a difference in electrophoretic mobility, was found to occur, together with the normal haemoglobin, as a trait in the non-sickling parents of these sick children. The discovery of this second abnormal haemoglobin, Hb C, implied at the same time that these children were heterozygous for the Hb S gene obtained from one parent and for the Hb C gene obtained from the other.

On account of haematological findings from non-sickling parents both Neel ('51) and Banks et al. ('52) took into consideration that interaction of the sickle cell gene with a thalassaemia gene caused clinical symptoms identical with those of sickle cell anaemia.

In Europe and in the U.S.A. such combinations had already been described though only among Caucasians of Mediterranean ancestry.[70, 62]

Electrophoresis of the haemoglobin of a negro patient from Banks' material, whose mother was non-sickling, proved the presence of 80 % Hb S together with haemoglobin of the same mobility as normal haemoglobin.[4] About the same time it was established that with sickle cell-thalassaemia, besides such an excess of Hb S, normal adult as well as foetal haemoglobin can occur.[57] The diagnostic significance of this finding, the pattern S + A+ F or S + A, was brought forward in 1955 in connection with the investigation of four negro families, where for the first time it was reported that sickle cell thalassaemia "may appear as a mild disorder, hardly causing any discomfort to the afflicted individual".[73] It has since been found that with this mode of interaction a S + F pattern

is also possible in which the presence of Hb A can not be established.[76]

Examination of a kindred, still resulting from Neel's material ('51), revealed another type of sickle cell — thalassaemia, in which the quantitative relationship between normal and sickle cell haemoglobin was not disturbed as in the above-mentioned cases.[9] This indicated the existence of two types of sickle cell — thalassaemia, described as "interacting" and "non-interacting".

A third form of interaction of the Hb S gene among negroids which attracts the attention in connection with the present study — although not arising from the family investigations already mentioned — concerns the persistent high foetal haemoglobin anomaly.

In 1955 Edington and Lehmann described for the first time the occurrence of high percentages of alkali-resistent haemoglobin in the presence of an excess of Hb S in two apparently healthy non-anaemic Gold Coast Africans.[14] They also found high Hb F values in the presence of normal adult haemoglobin in the children of these men.[15]

Jacob and Raper made four similar observations in Uganda in 1958 regarding non-anaemic individuals. As a result of family studies they considered "the anomaly as a genetically determined persistence into adult life of the foetal mode of haemoglobin synthesis".[35]

Unlike thalassaemia, persistent high Hb F and Hb C have only been encountered in individuals wholy or partly of negro descent.

The occurrence of Hb S as well as Hb C seems to be controlled by single genes which, according to genetic behaviour, are related as alleles or closely linked genes. Biochemical research concurs herewith. Also the anomaly resulting in the persistent high Hb F behaves as if its presence is due to the effect of a single gene.

With regard to thalassaemia, observations concerning the mode of inheritance together with physico-chemical and haematological findings — to be presented in the next chapter — lead one to accept the existence of more "responsible" genes.

To understand the interrelationship of these haemoglobinopathies clearly, the haemoglobin distribution among the sibships of marriages involving one partner heterozygous for two haemoglobin abnormalities and one "normal" partner, is of particular importance. Children with normal haemoglobin only or solely with both abnormalities together, are an argument against the presence of allelomorphic genes.[68]

Evaluation of findings among the sibships of twelve marriages where one of the partners was simultaneously heterozygous for the Hb S gene and for thalassaemia while the other partner possessed normal haemoglobin, pointed to the existence of two thalassaemia genes one of which was and the other was not allelic with respect to the Hb S gene.[68] In this connection it is important that the four negro families among this mate-

rial also showed the presence of allelic and non-allelic thalassaemia genes.[8, 9]

Family investigations, showing persistent high Hb F together with Hb S[22, 48, 77] or with Hb C[47, 85], proved that this anomaly appears to be determined by a factor allelic or closely linked with the genes for Hb S and Hb C.

The multi-family research on the occurrence of Hb S was mostly carried out to obtain sufficient quantitative data about the mode of inheritance of the Hb S gene itself. By using the sickle cell phenomenon as a phenotypic recognition mark in multi-family research, it is also possible to trace haemoglobinopathies which do not cause morbid symptoms in combination with Hb S.

CHAPTER III

PHENOTYPIC FEATURES OF THE Hb S AND SOME RELATED Hb GENES.

In the present study the genetic relationship of haemoglobin S with haemoglobin C, persistent high foetal haemoglobin and thalassaemia is of special interest. A summary should accordingly be given of modern opinion resulting from the development of biochemical genetics, after which the physico-chemical and haematological details of these haemoglobinopathies will be set out.

Analysis by new methods, the most important of which are electrophoresis, chromatography and alkali denaturation, shows that haemoglobin as found in a normal person is composed of a main Hb A_1 fraction and two smaller Hb A_2 and Hb A_3 fractions, whilst a very small amount of foetal haemoglobin may also be present.

The haemoglobin molecules of these fractions are made up of two pairs of polypeptide chains. The two chains in each pair are identical. The chains of one of the pairs, the α-chains, are found in all the fractions. The chains in the other pair are not the same for all the fractions (β-, δ-, and γ-chains in Hb A_1, Hb A_2 and Hb F molecules respectively). This indicates that the formation of the haemoglobin molecules of these fractions is regulated by four different genes, each of which controls the formation of one sort of polypeptide chain.

The fact that hereditary variations are known of each of the four chains supports the hypothesis that separate genes control the synthesis through α-, β-, γ- and δ-chains. Since α-chains form part of all haemoglobin molecules, a mutation producing a change in the α-chain would result in abnormal Hb A_1, Hb A_2 and Hb F. A mutation in the β-chain, on the other hand, would manifest itself in the Hb A_1 molecules whilst the synthesis of the Hb A_2 and Hb F molecules would not be affected "qualitatively", although quantitative changes of a compensatory nature can occur in the ratios between the fractions.[45]

The hereditary variations known as abnormal haemoglobins can be distinguished from the normal, and also as a rule from each other, by a difference in electrophoretic behaviour in that in many cases the replacement of one amino acid by another is coupled with a distinct charge difference. The rate of synthesis of an abnormal haemoglobin is as a rule slower than that of normal haemoglobin, and this is brought out by the normal-abnormal haemoglobin ratio as found in heterozygosity.

Ingram has shown that there is a variation in the β-chain in Hb S. In

this case glutamic acid, the sixth amino acid in the β-chain of normal haemoglobin calculated from the fourth terminal residue, is replaced by valine. The mutation which produces Hb C concerns the replacement of this glutamic acid by lysine.[32, 31, 34]

Similar replacements of one amino acid by another are known in the α- and δ-chains as well. These are also coupled with a difference in electrophoretic mobility.

Thalassaemia cannot be distinguished in this way as "another" abnormal haemoglobin. It is assumed that a suppression in the rate of synthesis in one of the chains is the primary effect of thalassaemia and is expressed by variations in the ratios between the haemoglobin fractions. The appearance of Hb H and Hb Bartholomew's haemoglobins, made up of four β-chains and four γ-chains respectively, also indicates an excess of these chains owing to suppression of the α-chain synthesis.[45]

As well as the α-thalassaemia, which gives rise to the formation of H and Bart's haemoglobins, there is also the frequently observed β-thalassaemia. An increase of the Hb A_2 fraction and a rise in the foetal haemoglobin indicate the presence of β-thalassaemia, which is presumed to effect suppression of the β-chain synthesis. Many β-thalassaemia trait carriers have an increased A_2 fraction which may or may not be coupled with an increase in the foetal haemoglobin. It is also possible that abnormalities of the erythrocytes may be the only indication of thalassaemia, whilst thalassaemia may even be completely indiscernible in the heterozygote, although its presence has to be assumed on the grounds of findings among family members.

These varying phenomena make it probable that there are several thalassaemia genes, and this is borne out by the findings regarding the presence of thalassaemia with abnormal haemoglobins.

"Interacting" and "non-interacting" thalassaemia are accordingly recognized. In the first case the encounter with the Hb S gene produces a change in the haemoglobin ratio resulting in an excess of the abnormal haemoglobin, whilst in the second the presence of thalassaemia is not coupled with a change of that type and the normal-abnormal haemoglobin ratio remains the same or practically the same as in the simple trait of the abnormal haemoglobin.[68]

Since on one hand thalassaemia genes behave as allelic or closely linked with the Hb S gene and on the other they appear to segregate independently, these different modes of inheritance also indicate the existence of more than one thalassaemia gene.

What is known of the synthesis of the haemoglobin molecules leads one to suppose that the anomaly known as persistent high foetal haemoglobin regulates the persistence of γ-chain production and a suppression of the

β-chain formation.[29, 77] The fact that when persistent high Hb F and Hb S are present together no Hb A can be shown whatsoever makes it probable that the suppression of the β-chains will be complete. The mode of inheritance makes it likely that this is determined by a gene that is allelomorphic or closely linked with the Hb S and Hb C gene.

PHYSICO-CHEMICAL AND HAEMATOLOGICAL FEATURES.

HETEROZYGOUS AND HOMOZYGOUS STATES.

Haemoglobin S.

Heterozygosity for the Hb S gene is accompanied as a rule by the occurrence of the sickle cell phenomenon under favourable circumstances only. The blood picture does not show abnormalities. There is no anaemia. In the presence of normal Hb A_1 the amount of Hb S ranges from 22 to 45%.[88] The Hb A_2 quantity is normal (approx. 2%) depending on the method of evaluation.

Homozygosity for the Hb S gene. Here the sickle cell phenomenon can be established more clearly. While as a rule a haemolytic anaemia consists of 5 to 8 gr%, the blood film is characterized by the presence of sickle cells, by anisocytosis, poikilocytosis, and by polychromasia. Normoblasts and target cells may occur. The osmotic fragility is usually moderately lowered.[12, 88] Hb S alone can be present, but in most cases about 5 to 12% Hb F will also occur. The presence of 24% Hb F has been observed.[88] The Hb A_2 value is normal but sometimes appears to be raised.

Haemoglobin C.

Heterozygosity for the Hb C gene is characterized by an increased number of target cells. There is no anaemia. In the presence of Hb A_1, Hb C is observed in quantities of 25 to 40%.[88]

Homozygosity for the Hb C gene is accompanied by the presence of many target cells in the blood film. There is generally but not always a moderate anaemia. In the presence of Hb C, a few per cent Hb F also occurs. The amount of Hb A_2 is raised.[37]

Thalassaemia.

Heterozygosity for thalassaemia, the "classical" thalassaemia that is, is characterized by microcytic polycythemia, anisocytosis, ovalocytosis and also by hypochromia and punctate basophilia, while the number of target cells can be increased. The osmotic fragility is distinctly lowered. The usually mild anaemia is refractory to iron therapy.

Heterozygosity for thalassaemia in general is most often accompanied by Hb A_2 increase,[18,40, 42] with or without Hb F increase, although either or both can be absent. The absence of Hb A_2 increase was relatively frequently established in negroids.[9] Besides the typical morphological characteristics described above, less distinct signs have been observed in persons of negro descent.[75]

In the West Indies classical thalassaemia with typical morphology, polycythemia and a high Hb A_2 level was found in persons of "pure" Negro stock, by Went and MacIver in Jamaica.[86]

In one among ten negro family members with thalassaemia minor (a mother of two children with thalassaemia major) the red cell morphology was not strikingly abnormal, although there was some microcytosis, while the osmotic fragility was normal.

During an investigation among 93 healthy female West Indian University students (in whom there is a higher proportion of Caucasian blood than among the majority of the Jamaican population) two cases of classical thalassaemia minor were found. In contrast the third case showed increase of Hb A_2 and an elevated Hb F level, but no morphologic abnormalities. It was concluded that "despite this there can be little doubt that the girl has thalassaemia minor".

Homozygosity for "classical" thalassaemia is accompanied by a characterized hypochromia, anisocytosis including micro- as well as macrocytosis, poikilocytosis, normoblastosis, sometimes polychromasia and punctate basophilia. The osmotic resistance is raised. Severe anaemia is present.[12, 88] The quantity of Hb F is considerably raised, while Hb A_1, although present in most cases, can be totally lacking. The Hb A_2 is not raised.

Persistent high Hb F.

Heterozygosity for the persistent high Hb F anomaly does not show any morphological peculiarities in the blood film, and is not accompanied by anaemia. The osmotic resistance is increased. In the presence of Hb A_1 the amount of Hb F ranges from 25 to 40 %. The quantity of Hb A_2 is low normal.[29]

Homozygosity for the persistent high Hb F anomaly has only been observed once up to the present. The presence of Hb F only was established.[29]

DOUBLE HETEROZYGOUS STATES.

Heterozygosity for the Hb S and Hb C gene. Here the sickle cell phenomenon occurs readily. The presence of many target cells, more than 50 %, is a typical feature. The osmotic fragility is diminished. Moderate anaemia can be present, but can also be lacking. As an exception a sporadic sickle cell may be observed in the blood film. Hb A_1 is not present. In the presence of Hb S the amount of Hb C ranges from 50 to 67 %. The amount of Hb F can be a few per cent or nil.[88]

Heterozygosity for the Hb S gene and thalassaemia.

"Interacting" type: the sickle cell phenomenon can easily be observed. The blood picture is characterized by microcytosis, anisopoikilocytosis and hypochromia, and ovalocytosis, many target cells and sickle cells as well. The osmotic resistance is clearly increased. The anaemia can be moderate or severe. The quantity of Hb S is 60 to 80 %. In the presence of Hb S, Hb A_1 as well as Hb F can occur together or separately. In the presence of both Hb A_1 and Hb F up to 17 % of Hb F has been established. As a rule Hb A_2 is raised.[12, 88]

"Non-interacting" type: the number of observations remained limited. The sickle cell phenomenon is present and the quantities of the Hb A_1 and Hb S fractions here are equal to those of the Hb S trait. However, the osmotic fragility is diminished.

In an American family the blood pictures were characterized by aniso- and microcytosis, more or less definite hypochromia, many target cells, and by ovalocytosis in the presence of a moderate anaemia. Hb S was observed in quantities ranging from 22 to 36 %. Hb F was not raised. Hb A_2 was normal, also in the family members showing the thalassaemia trait only.[9]

Among the Eti-Turks in South Turkey two types of sickle cell-thalassaemia were reported, an "interacting" one and another resembling the "non-interacting" type. The latter was characterized by an increased number of target cells and by a moderate anisocytosis, while Hb F was present up to about 10% or else absent. The slight anaemia was micro- to normocytic.[2] The occurrence of both types in twins was reported separately.[1]

Heterozygosity for the Hb S gene and the persistent high Hb F anomaly.

The formation of sickled erythrocytes is either difficult to observe [29] or else may be observed more or less immediately.[22] The blood picture shows either no peculiarities or else an increase of the number of target cells. There is no anaemia. The osmotic resistance is increased. In the presence of Hb S the amount of Hb F ranges from 25 to 40 %. Hb A_1 is totally absent, while the amount of Hb A_2 is low normal.[29]

CHAPTER IV

THE FITNESS OF HAEMOGLOBIN S CARRIERS.

The word "haemoglobinopathies" implies that the existence of these abnormalities was first brought to light by the study of clinical syndromes. Zuelzer viewed it as of "more than theoretical interest" that this should be borne in mind and considered that "at least some haemoglobins should be looked upon as merely variants like blood groups and skin pigments, and that their presence confers no handicap".[87]

In the tropics, where these aberrant haemoglobins occur most frequently and the carriers sometimes form a large part of the population, it is important to establish what effect these hereditary factors have on the carriers' general health.

The fitness of these persons is an obvious point to be considered. This occurs particularly where the accent is one-sidedly determined by the homozygous forms of sickle cell haemoglobin and also of thalassaemia which produce serious clinical syndromes at an early age.

As far as could be ascertained, the fitness of Hb S carriers had never previously been an object for study.

A longer period of study is generally desirable in order to gain an idea of the fitness of these carriers. For obvious reasons this aspect did not arise in the tropics, where the period of observation is usually limited. It must also be possible to assess both asymptomatic and symptomatic abnormal haemoglobin carriers in the same manner.

Fitness is determined by the general bodily condition and the way in which ailments are experienced and undergone. Despite subjective factors, an idea of fitness can be obtained by studying the disease and ailment frequencies coupled with observation of bodily condition among a given number of persons with the same abnormality. The results of a study of this sort will be affected by the period and the type of community in which those concerned live and will be comparable with data obtained in a similar way for persons with similar abnormalities living under the same circumstances.

An evaluation of this type is of importance both socially and medically. In view of the increasing interest in Hb S trait carriers initially assumed to be asymptomatic, it is logical to establish the significance of the incidental clinical observations in this respect.[46, 66] As increasing industrialization in tropical areas is to be expected, it is reasonable to check whether the exertion called for by industrial processes might not exceed the capacities of adult haemoglobinopathy carriers. It is also in the interests

of others that the risk of industrial accidents, for instance, should not be raised unnecessarily.

In the "natural histories" of sickle cell anaemia in children both in Africa [78] and America,[20] and the studies on haemoglobin SC disease in children [79] and adults,[24, 26, 67] more attention has been paid to the history of the disease as such than to the fitness of the carriers, as is after all inevitable in view of the clinical approach followed.

Since the morbid syndromes as observed on Curaçao have already been described in the pre-Pauling period and after, [6, 84] this study will be concerned with frequency data of diseases and ailments recorded for Hb S carriers in connection with this haemoglobinopathy, being inspired in this aim by Rucknagel and Neel's remark: "unfortunately, in much of the literature lack of electrophoretic documentation and family studies make an estimate of the relative frequency of these complications in the various sickle cell variants impossible".[68]

PART II

INVESTIGATION

CHAPTER V

MATERIAL

SELECTION

The collection of the haemoglobin material was based on the results of the sickle tests according to Emmel as applied and reviewed during the workers' periodical medical examinations (since 1943), and during examinations, including pregnancy checks, of their wives (since 1949).

This method of approach is governed by:

- the use of the sickle cell test as a screening method;
- the indications calling for this test;
- the men and women concerned.

This screening method* cannot be considered as an objective approach to sickle cell haemoglobin. However, as regards the indications calling for the sickle cell test and as far as the periodical checks are concerned, the approach may be described as "non-clinical".

Men and women were selected through the employment relationship of the men or the partners of the women with the oil industry. The men's ages are accordingly determined by the working period of their lives.** It should also be borne in mind that in previous years men had been rejected on medical grounds, in some cases either knowingly or unknowingly owing to the presence of abnormal haemoglobins.

As a result of this possibility certain haemoglobin combinations might be missing among the workers now being examined. The presence of the sickle cell phenomenon in itself was no reason for rejection. As for the women, only workers' wives of a certain age group were examined.

The possibility of relationship among the men and women concerned could not be excluded.

ORIGIN OF THE MEN AND WOMEN EXAMINED

Sufficient men and women, all negroids and originating from the undermentioned regions, were examined for the presence of the sickle cell phenomenon to determine the number of positive and negative results of their tests.

Curaçao	Surinam	Saba	Nevis	Dominica	Grenada
Aruba	St. Eustatius	Anguilla	Antigua	St. Lucia	Barbados
Bonaire	St. Maarten	St. Kitts	Montserrat	St. Vincent	Trinidad

* The limitations of the sickle cell test will be treated under the investigation methods.
** Up to 60, the retirement age.

The number of men and women tested for sickle cells was:

age between	*men (17–60)*	*women (15–69)*	*total*
Curaçao	3097	1606*	4703
all other regions together	1225	916	2141
total	4322	2522	6844

Haemoglobin material was ascertained by delimitation using positive results only.

HAEMOGLOBIN MATERIAL

The haemoglobin material covered samples from:
- the "sickling" men and women;
- family members ascertained through the presence of the sickle cell phenomenon in the blood of one or both of the parents.

Haemoglobin from 433 sickling persons (workers and women) was examined by electrophoresis.

age between	*men (18–60)*	*women (15–61)*	*total*
Curaçao	200	91**	291
all other regions together	80	62	142
total	280	153	433

Haemoglobin from family members of 127 of these sickling men and from 111 of these sickling women was examined in the same way.

222 families were involved, out of which one of the parents in 206 families had been found to be a sickle cell carrier whilst in 16 families both parents had shown the phenomenon.

This part of the investigation included:

206 haemoglobin samples from non-sickling partners; 1124 samples from children.

The whole material included 1763 haemoglobin samples (280 from workers; 153 from women; 1330 from family members).

*1393 women, aged between 15 and 50 years, were examined for the presence of the S phenomenon during pregnancy checks.

** 75 women, aged between 15 and 50 years, were found to be sickling during pregnancy checks.

The co-operation of Dr. Carlos Winkel (chief) and Dr. N. J. Jansonius of the paediatric department of the St. Elisabeth Hospital in Willemstad made it possible to extend the family investigation as far as children were concerned. This supplementary research was guided by recognized or suspected pathology in polyclinical and clinical patients, and carried out after a positive result of the sickle cell test had been established for one of the children.

Twelve families were investigated, amounting to the haemoglobin samples of 24 parents and of 72 children.

In all, 1859 samples were examined by electrophoresis for this study.

CHAPTER VI

METHODS

GENERAL

In choosing methods for the investigation the quantity of the genetic material had to be taken into account. Paper-electrophoresis, generally recognized as being a simple and most useful qualitative technique for investigating larger quantities of haemoglobin samples[5, 28, 88] was introduced as a basis for the study.

This method could be applied to haemoglobin material which had been selected by using the positive results of sickle cell tests only.

This approach has to be considered as the presence of Hb S is not always accompanied by the appearance of the sickle cell phenomenon.

a) Haemoglobin samples with a Hb S concentration below the minimum required for this phenomenon, may have been missed. However the occurrence of Hb S in such a low concentration must be considered exceptional, this being reported only once[72], in contrast to many observations proving Hb S usually to be present in an amount of 20 to 45%, in cases of sickle cell trait.[88]

b) It may be difficult to obtain the formation of sickled erythrocytes in cases of heterozygosity for Hb S as well as the persistent high Hb F anomaly[29], although this has not been general experience.[22]

By testing each haemoglobin sample from sickle cell positive blood by paper-electrophoresis, it was possible to confirm the presence of S haemoglobin and eliminate "false" positive results of the sickle cell test. Haemoglobin electrophoresis was also applied for the supplementary research on the families concerned. The combined positive results of this haematologic and electrophoretic investigation have been accepted as proof of the presence of S haemoglobin, since the sickle cell phenomenon is a unique and specific morphologic indication of its presence, whilst differentation of sickle cell haemoglobin is not necessary as up till now all S haemoglobin samples from different parts of the world have proved to be identical.[33]

The interpretation of results obtained by paper-electrophoresis of single haemoglobin samples is limited.

Patterns resulting from double heterozygosity for Hb S and "non interacting" thalassaemia are indistinguishable from those of the simple sickle cell trait.[58] Moreover full scale family research is often necessary to confirm or exclude the presence of "interacting" thalassaemia or persistent high Hb F in the case of SF patterns.

As simultaneous family research could be carried out where required, genotypic interpretation of the haemoglobin patterns was made possible, with the exception of "non-interacting" thalassaemia.

The S+A+F and S+F patterns obtained made the application of other laboratory methods desirable and also brought out the need for a simple screening and routine technique for quantitative determination of the Hb A_2 fraction when no laboratory specialized in haemoglobin analysis is available.

A parallel investigation into the practical possibilities of a Tris-hydroxymethylamino-methane buffer resulted in the development of a discontinuous Tris buffer paper-electrophoresis for semi-quantitative determinations by Dr. E. D. A. Sindram, head of the Public Health Laboratory at Willemstad.

Thanks to this co-operation the application of alkali denaturation, Amberlite IRC-50 chromatography, and determinations of serum iron were possible during examinations of families in which Sickle cell-thalassaemia and Sickle cell-persistent high Hb F had been found.

At the same time haematological investigation of the people concerned took place in the Green Cross Hospital laboratory at Willemstad.

Blood group determinations (A, B, O, and rhesus system) were carried out in each case to check the families examined.

Screening method

The moist cover slip test according to Emmel is the most simple method of observing the sickle cell phenomenon. The typical transformation of the erythrocytes is due to the formation of intracellular semi-crystalline aggregates when the oxidized sickle cell haemoglobin passes into the reduced state.[21]

As has already been mentioned, a minimum of intracellular concentration of S haemoglobin is essential. "False" sickle cells may occur.[69]

Using this simple technique a drop of fresh blood is sealed from the atmosphere by paraffin between a slide and a cover slip. This is then examined under a microscope several times during 3 x 24 hours.

Preparation of haemogobin solutions

Immediately after venapuncture 10 cc blood is mixed with 1 cc 1% heparine solution in normal saline. The mixture is centrifuged for 10 minutes at 2000 r.p.m. The plasma together with the grey white upper layer of the packed erythrocytes is removed and the erythrocytes are washed carefully three times, each time with a three-fold volume of normal saline. After each washing they are centrifuged for 10 minutes at 2000 r.p.m. The salt solution is then removed and an equal volume of distilled water and a half volume of toluene is added to the erythrocytes.

After being shaken vigorously for 1 to 2 minutes, the mixture is kept for at least 12 hours at a temperature of 0 to 4° C.

It is then centrifuged for 30 minutes at 4000 r.p.m. The toluene, the stroma and the upper layer of the haemoglobin solution are removed and one drop of a watery merthiolate solution (1 : 1000) is added to the clear wine-red haemoglobin solution obtained.

Qualitative methods

Zone electrophoresis on paper is carried out as a comparative method for haemoglobin analysis. Its principle is based on differences in the charges of the various haemoglobins at a selected acidity of the buffer. Analysis is made possible by comparing the relative mobilities of the different haemoglobins in an electric field. Absolute mobilities cannot be measured when dealing with paper-electrophoresis because of electro-osmotic influences, evaporation and the inhomogeneity of the electric field in the paper.[64] However, for qualitative comparative analysis of haemoglobins this method is very useful. All analyses were made by applying horizontal electrophoresis in a "pressure plate" apparatus on Whatman 3 MM paper, using barbital buffer pH 8.6 and a 350-volt current.

For a description of the technique the publication by Smith and Conley should be referred to[74], and for the method applied in the Groningen paediatric laboratory see Jonxis and Huisman.[38] However, certain particulars of the present author's technique must be given.

The electrophoresis was carried out in an air-conditioned room at a temperature of 20-22° C without extra cooling of the apparatus; 5 or 7 haemoglobin spots were applied on each paper strip (width 13 cm). Each electrophoretic "run" lasted 11 to 12 hours.

The best results were obtained with silicon glass plates of 13 mm thickness, as these made it possible to distribute the pressure evenly *.

The strips were examined after drying for 10 to 15 minutes at 130° C in a horizontal position. The relative mobilities of the abnormal haemoglobins and the fractions of normal haemoglobin observed under the conditions described can be indicated as follows:

$$A_{2} > A_1 > F > S > A_2 > C$$

The main fraction of normal adult haemoglobin (Hb A_1) moved 6 to 7 cm from the starting line in the middle of the paper between the two buffer compartments. At the same time Hb F migrated 5 cm, Hb S 4 cm,

* In thinner plates the electrophoresis was affected by the flexibility of the glass. To silicon the plates a 3% solution of silicon oil DC 200, 10,000 ctks in tetrachlorine-carbon was used. After coating with this liquid the plates were heated for 24 hours at 160—170° C and the oil was then carefully removed with a cloth.

and Hb C 2 cm. In the case of an excess of haemoglobin A the presence of foetal haemoglobin could be guessed by a "tailing" effect at about 5 cm if at least 10% of Hb F was present. In the presence of an excess of Hb S haemoglobin F forms a triangular shape at about 5 cm, the base of which rests on the Hb S spot while its apex stretches to, and sometimes into, the location of Hb A_1. It was not possible to determine from the electrophoretic pattern in these cases whether or not there was also a small quantity of Hb A present.

No differentiation between the S+F and the S+F+A patterns can be made when Hb A is present in S+F+A mixtures in minute quantities. Other members of the family then have to be examined to determine the genotype of the haemoglobin.[44]

The normal Hb A_2 fraction could not be distinguished on the unstained strip. On the other hand the increased A_2 fraction could be, depending the quantity of the haemoglobin solution applied. However, in all cases of increases of the A_2 fraction during the electrophoretic "run" a "tailing" effect between the A_1 and the A_2 fraction could be observed, and this was independent of the quantity of haemoglobin used. At the end of the electrophoresis the "tailing" effect became less marked as the distance between the fractions increased and finally disappeared. This passing effect was unmistakable. A simultaneous analysis of a Hb A sample without A_2 increase was required for comparison. All Hb A_2 increases were observed in this way and afterwards confirmed by a quantitative method mentioned later.

Chromatography by the cuvette method is most useful for the comparative qualitative chromatography of haemoglobins and takes little time. Its uncomplicated apparatus is suitable for a large number of analyses. This chromatography is based on the different absorption rates of the various haemoglobins using kation exchange Amberlite resin IRC–50 as adsorbent and citrate buffer as elution solution.

For a description of the apparatus and technique see Prins's thesis[63] and the publication by Jonxis and Huisman.[38]

The relative mobilities of the observed zones of abnormal haemoglobins and normal adult and foetal haemoglobin can be indicated in order of decreasing mobility by:

$$F > A > S > C.$$

A good separation of Hb A and Hb F is obtained by chromatography in contrast to the bad one from paper electrophoresis. Judging from the results "leading" zones, preceding the main fractions, should be taken into account.[63, 64]

The chromatographic results obtained in the Public Health Laboratory, Curaçao, repeatedly showed a haemoglobin zone between the Hb S and the Hb F zone at the height of the Hb A zone running simultaneously in a secondary cuvette. This "intermediate" zone was observed in genetically confirmed Hb S homozygosity, in Hb S – persistent high Hb F, and in Hb S – thalassaemia. Thus the interpretation of "intermediate" zones was impossible without family research being carried out at the same time, as visual differentiation between S + A + F and S + s ("leading" zone) + F is frequently speculative. The zone between Hb S and Hb F could be interpreted as Hb A only when the electrophoretic pattern also produced a distinct S + F + A pattern. In these cases this zone possessed a higher intensity compared with the "leading" zone (s) of haemoglobin S.

Quantitative methods

Alkali denaturation is the most important method – from the practical application angle as well – for differentiating between the foetal haemoglobin and the adult haemoglobin.[27] It is based on the increased alkali resistance of foetal haemoglobin as described for the first time in 1866 by Körber.

With the spectrophotometric method of Jonxis and Visser 0.06 N NaOH is used and spectrophotometry is carried out at a wavelength of 576 mu.

The denaturation of foetal haemoglobin occurs as a mono-molecular reaction, and therefore the logarithms of the percentages of non-denaturized haemoglobin, calculated at different times, are situated on a straight line. The percentage of foetal haemoglobin in the original blood can be calculated by extrapolation. The original publication of Jonxis and Visser is referred to for a description of this technique as applied in the Public Health Laboratory, Curaçao, using a Beckman D.U. spectrophotometer.[39]

The values mentioned in this study are to be considered normal up to 1½% Hb F.

Discontinuous Tris buffer paper-electrophoresis (by Dr. E. D. A. Sindram)

This is a simple method for semi-quantitative determination of the A_2 haemoglobin fraction. In view of its apparatus, technique and supporting medium this method is appropriate for use when starch electrophoresis would be too cumbersome. The technique described by Goldberg is followed, based on the use of different buffers on the paper strips and in the buffer compartments.[19] Deviations from the original method are introduced to obtain the most suitable results when using the Spinco electrophoresis system and the Spinco scanner.

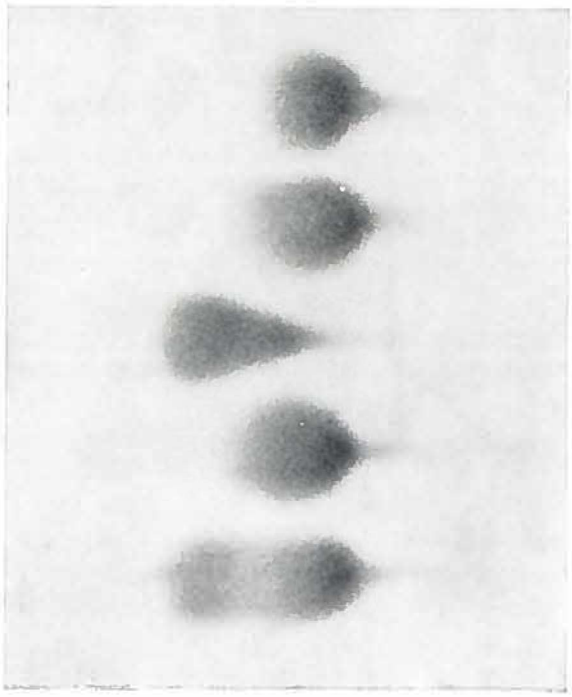

Fig. 1. Electrophoretic haemoglobin patterns (fam. V) From above: 1. Hb A (control); 2. Hb's A + persistent high F (father); 3. Hb's S + p.h. F (child 2); 4. Hb's A + p.h. F (child 1); 5. Hb's A + S (mother).

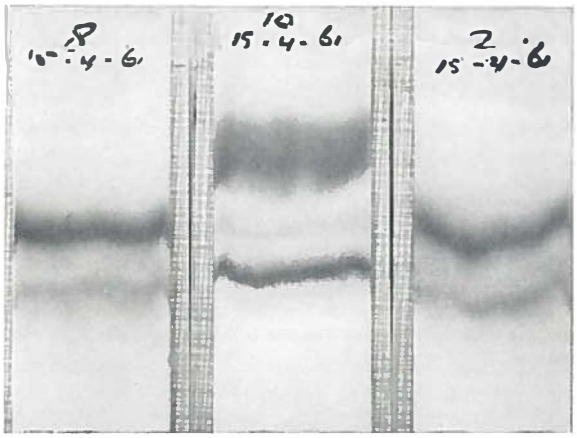

Fig. 2. Chromatography Amberlite IRC–50: "intermediate" zone in Hb's S + p.h. F and "leading" zone of Hb F (child V 2); in the other cuvettes: Hb's A + p.h. F.

The electrophoresis is carried out in the Spinco apparatus consisting of a "paper-electrophoresis cell, Durrum type, model R", and a "Duostat power supply", which is standardized and available in many laboratories. A Spinco "Analytrol" is used for quantitative determination. In the whole procedure the original A system* is followed which determines at the same time the choice of the supporting medium, Whatman 3 MM paper, and the staining technique to be discussed later.

Buffers:

1. "Tris" buffer, 0.12 molar, pH 9.1 :3
   6.050 g Tris-hydroxy-methylamino-methane (Sigma 121), 0.780 g disodium ethylene-diaminetetra acetate dihydrate, reagent grade (Complexon III) and 0.460 g boric acid, reagent grade, are dissolved in aqua bidistillata and adjusted to 500 ml.
2. Barbital buffer, 0.058 molar, pH 8.6 :
   30.90 g sodium barbital and 4.20 g diethyl-barbituric acid are dissolved in aqua bidistillata and adjusted to 3000 ml.

Procedure: The barbital buffer is poured into the buffer compartment and adjusted to the right level. Whatman 3 MM paper strips are dipped in Tris buffer, placed on filter paper for 1 minute, then introduced into the apparatus. The current is connected during aequilibration (15 minutes, current 5 mA per compartment, i.e. 0.21 mA per cm of paper). Per strip 0.0035 ml 10 % haemoglobin solution is applied.** Electrophoresis is carried out during 16 hours at room temperature (20–22° C). Immediately after the electrophoresis the strips are allowed to dry for 30 minutes at 120° C., during which they should be well ventilated.

Staining method: The paper strips are stained for 6 hours in a bath containing 100 mg bromium-phenol blue, 31 g. sulphate of zinc (1 aeq.) and 50 ml 95% alcohol made up to 1 litre with 5 % acetic acid.

The strips are then put into two successive baths of 5 % acetic acid for 2 x 6 minutes and finally placed for 6 minutes in a fixing bath of 9.0 g sodium acetate (3 aeq.) in 1 litre of acetic acid and then allowed to dry for 20 minutes at 120° C. The strips are placed in NH_3 vapour for 1 minute before quantitative interpretation is begun.

Conditions: The electrophoretic strips and the curves have to meet certain conditions in order to obtain a useful result. The migration of the haemoglobin pattern should be less than ½ cm from the starting line in the direction of the cathode. The correct width of a normal pattern (measured from the point of application) has to be about 6 cm.

The use of the quantity indicated of an exactly 10 % haemoglobin solution is essential to obtain a distinct peak of the Hb A_1 in the curve.

* The original system introduced by Beckman, Spinco devision. The same Company later developed the (R) system, involving modifications of the paper-material and staining technique used and of the use of the "Analytrol" scanner.

** After the haemoglobin solution is prepared 0.025 ml is mixed with 5 ml water and checked in a Klett photo-colorimeter (filter 54). The colour intensity is measured on a standard curve obtained from known haemoglobin solutions, after which it is diluted to exactly 10%.

For correct calculation of the A_2 value the peak of the Hb A_1 curve must be situated at the level of the 11 cm line on the graphic paper (Beckman 300–542).

Application of a larger quantity of haemoglobin results in a higher peak or an extension of the Hb A_1 curve, giving rise to a disproportionate increase in the A_2 value.

Interpretation of the curve: By placing the electrophoretic strip above the curve the middle of the A_2 curve is determined as accurately as possible. The A_2 curve is then drawn in full together with the most probable separation between the A_1 and A_2 curve. The staining substance between Hb A_1 and Hb A_2 in the electrophoretic pattern is assumed to consist of Hb A and possible impurities due to "tailing". In the presence of the A_2 fraction more of the proteins remain behind than when Hb A_2 is absent.

A Hb A solution without the A_2 fraction does not rise above the normal Hb A_1 tailing-curve at the A_2 position. Also low A_2 values indicate a lower quantity than do high A_2 values.

If one considers the whole curve of an electrophoretic pattern of normal adult haemoglobin (fig. 3), it can be seen that there is a small rise close to the point of application which can in some cases be more pronounced and sometimes reaches the height of the A_2 peak. This is the point where proteins which are not haemoglobins and the B_2 fraction should be found.[25] However, owing to the staining technique applied they cannot be distinguished from each other. After this "threshold" rise the curve passes a minimum. The height of this minimum is of the same order of magnitude as the deflection of the tailing curve of a "pure" Hb A_1 solution.

This was found from tests where immediate electrophoresis of this fraction was carried out after electrophoresis of a number of normal haemoglobin solutions and isolation of the Hb A_1 fraction obtained while the strips were still wet (fig. 4).

On account of these findings it is assumed that a "tailing" with the height of the minimum, as mentioned, belongs to the Hb A_1 fraction in each electrophoretic strip. When calculating the A_2 fraction the height of this minimum is taken as base and only the A_2 curve surface above this minimum is calculated. The surface below this base line up to the point of application is added to the Hb A_1 curve-surface.

The A_2 fraction can also be determined from electrophoretic A + S (S trait) patterns. However, as the A_2 fraction does not entirely separate out of the Hb S fraction a separation between these two is more difficult to establish. Principally the calculation is done in the same way, bearing in mind that the surface below the base line is added to the joint Hb A and Hb S curve-surface.

An exact separation between the A_2 fraction and the Hb S fraction may be very difficult if a predominant quantity of Hb S is present in the electrophoretic pattern.

Results. The height of the base line is of great importance for the calculation of the A_2 curve-surface but not so for calculating the Hb A_1

curve-surface. The determination of this base line is an important cause of differences in results. Furthermore, despite the careful selection of the paper strips the varying properties of these undoubtedly affect the curve.

Spreading of the results, up to 1 % haemoglobin, is due to these imperfections. Consequently a single incidental test on one strip is not sufficient to obtain a good quantitative impression.

The low normal and the distinctly increased A_2 values are immediately recognized as such (fig. 5).

To be certain in cases of doubt or to obtain a semi-quantitative approximation, several patterns from each sample have to be made. However, by preparing 8 electrophoretic strips of a haemoglobin sample (completely filling the electrophoresis apparatus) average A_2 values are found which are reproducible up to a ½ %, and can be compared with the results obtained by other methods.[10, 23]

To obtain the normal average A_2 percentage, A_2 values were determined from 50 samples (100 strips) of haemoglobin A and from 25 samples (50 strips) of haemoglobin A + S from members of those families which did not show indications of thalassaemia when screened.

The values obtained are:

Connected with Hb A : 2.4 % of A_2; spreading 0.8—3.5 %

Connected with Hb A + S : 2.6 % of A_2; spreading 1.0—3.3 %

The average normal A_2 value was also calculated from 218 individually examined Hb A samples which did not show increased A_2 fractions during qualitative electrophoresis. The value obtained is 2.2 % of A_2, spreading 0.8—3.5 %, standard deviation 0.62.

From the results of these observations the maximum normal value of A_2 for the method described is set at 3.5 %.

Examination elsewhere* of the various haemoglobin solutions found to have critical values around the maximum normal percentage, gave fairly similar results.

In the presence of clearly increased A_2 values this technique yielded higher results (up to a few per cent) than those obtained elsewhere.

Haematological methods.

Haemoglobin concentrations were determined by the cyan methaemoglobin method using a Klett-Summerson photocolorimeter (reagents and standard by Ortho Pharmaceutical Corp., New Jersey).

Erythrocytes were counted in the usual way and reticulocytes counted as described by Muller and Verschure.[49]

Blood films were stained according to Leishman.

* Prof. T. H. J. Huisman (Medical College of Georgia, Augusta, U.S.A.).

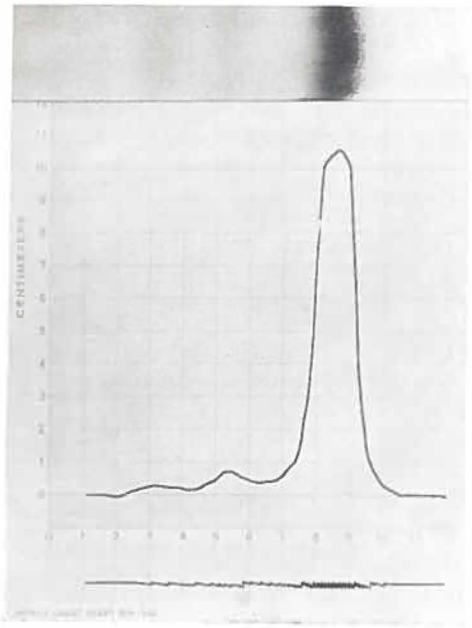

Fig. 3.

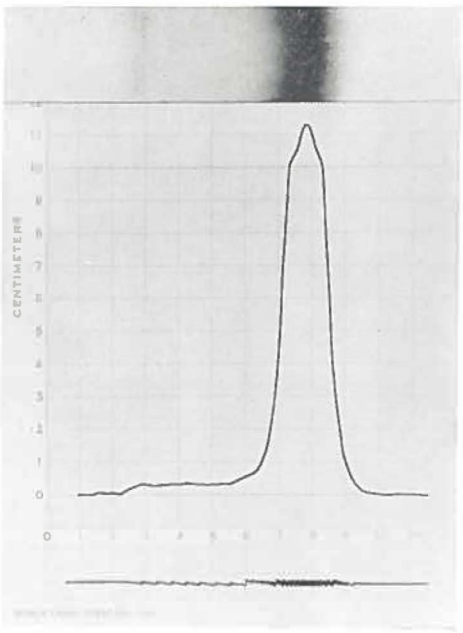

Fig. 4.

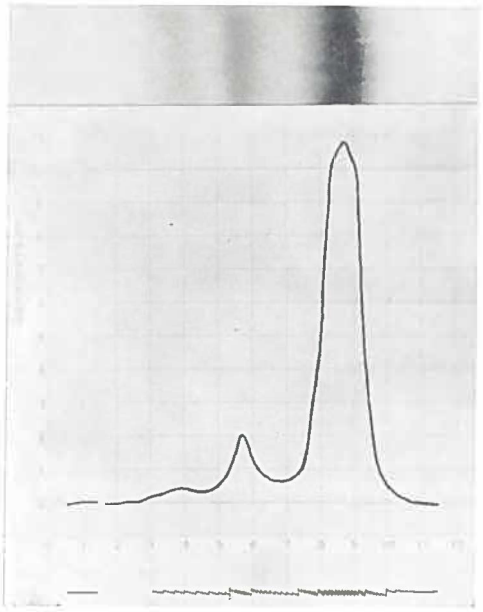

Fig. 5.

The packed cell volume was determined as described by Van Allen,[80] and the osmotic fragility by the Dacie method[11] (room temperature 20–22° C). No simultaneous controls of normals were possible.

The plasma bilirubin concentration was determined by the usual method, total serum iron by the method described by Simmons and Gentzkow,[71] and the total iron binding capacity according to Ramsay.[65]

CHAPTER VII

RESULTS OF THE INVESTIGATION

1. THE FREQUENCY OF THE Hb S TRAIT AND THE OCCURRENCE OF Hb S TYPES AMONG MEN AND WOMEN.

a. *Curaçao workers.*

A total of 3097 Curaçao workers aged between 17 and 60 were examined for the presence of the sickle cell phenomenon.

203 of these proved to be sickling.

It was possible to examine the haemoglobin of 200 of these men (three aged 57, 58 and 59 respectively were not available for the examination).

On the basis of electrophoretic haemoglobin patterns heterozygosity for the Hb S gene could be assumed for all men examined. Consequently the frequency of the Hb S trait among the men examined for the presence of the sickle cell phenomenon is 6.6 % (heterozygosity is assumed in the three sickling men where the presence of Hb S was not confirmed by electrophoresis).

The following electrophoretic patterns were obtained:

A + S(192), S + C (6) and S + A + F (2)

The two individuals possessing S + A + F patterns were related (brothers).

TABLE 1 – *The frequency of the Hb S trait in relation to the age of the Curaçao workers.*

age group	20 – 29	30 – 39	40 – 49	50 – 59	20 – 59
number S tests	1172	840	538	370	2920
pos. tests	73	50	46	27	196
% S trait	6.2	5.9	8.6	7.3	6.7

The false positive sickle cell tests were taken as negative and as such form part of the total number of S tests (= 100.1 %).

TABLE 2 – *The frequency of the Hb S types in relation to the age of the Curaçao workers.*

age group	20 – 29		30 – 39		40 – 49		50 – 59		20 – 59	
Hb pattern	%	number	%	number	%	number	%	number	%	number
AS	6.0	(70)	5.5	(46)	8.4	(45)	6.5	(24)	6.3	(185)
SC	0.1	(2)	0.3	(3)	0.2	(1)	–	–	0.2	(6)
SAF	0.1	(1)	0.1	(1)	–	–	–	–	0.1	(2)
no exam.	–	–	–	–	–	–	0.8	(3)	0.1	(3)

b. *Curaçao married women.*

The total number of Curaçao married women aged between 15 and 69 examined for the presence of the sickle cell phenomenon was 1606.

95 of these proved to be sickling.

The haemoglobin of 91 women was examined electrophoretically (four women aged 25, 31, 44 and 52 respectively were not available for examination).

On the basis of electrophoretic haemoglobin patterns heterozygosity for the Hb S gene could be assumed for all women examined. The frequency of the Hb S trait among the women examined for the presence of the sickle cell phenomenon is 5.9 % (heterozygosity assumed in four women without confirmation by electrophoresis).

The following electrophoretic patterns were obtained:

A + S (89) and S + C (2)

TABLE 3 – *The frequency of the Hb S trait in relation to the age of the Curaçao women.*

age group	20 – 29	30 – 39	40 – 49	50 – 59	20 – 59
number S tests	597	566	294	116	1573
pos. tests	37	23	21	11	92
% S trait	6.2	4.1	7.1	9.5	5.8

TABLE 4 – *The frequency of the Hb S types in relation to the age of the Curaçao women.*

age group	20 – 29		30 – 39		40 – 49		50 – 59		20 – 59	
Hb pattern	%	number	%	number	%	number	%	number	%	number
AS	5.9	(35)	3.9	(22)	6.5	(19)	8.6	(10)	5.5	(86)
SC	0.2	(1)	–	–	0.3	(1)	–	–	0.1	(2)
no exam.	0.2	(1)	0.2	(1)	0.3	(1)	0.9	(1)	0.2	(4)

1374 women aged between 20 and 50 were examined for the presence of the sickle cell phenomenon during pregnancy checks.

74 of these women showed a positive sickle cell test.

The haemoglobin of 71 women was examined by electrophoresis.

TABLE 5 – *The frequency of the Hb S trait in relation to the age of Curaçao women examined during pregnancy checks.*

age group	20 – 29	30 – 39	40 – 49	20 – 49
number S tests	549	541	284	1374
pos. tests	33	22	19	74
% S trait	6.0	4.1	6.7	5.4

TABLE 6 – *The frequency of the Hb S types in relation to the age of Curaçao women examined during pregnancy checks.*

age group	20 – 29	30 – 39	40 – 49	20 – 49
Hb pattern	% number	% number	% number	% number
AS	5.6 (31)	3.9 (21)	6.0 (17)	5.0 (69)
SC	0.2 (1)	— —	0.4 (1)	0.2 (2)
no exam.	0.2 (1)	0.2 (1)	0.4 (1)	0.2 (3)

c. *Persons originating from other Caribbean areas.*

The number of persons aged between 20 and 60 originating from other Caribbean areas examined for the sickle cell phenomenon was 2141 (1225 men and 916 women).

149 (81 men and 68 women) were found to be sickle cell positive. The haemoglobin of 142 of these persons (80 men and 62 women) was examined (one man and six women were not available for the examination).

On the basis of electrophoretic haemoglobin patterns heterozygosity for the Hb S gene could be assumed for all persons examined, with one exception to be commented on later.

The frequency of the Hb S trait was not calculated separately for each region as the number of individuals examined was small. In table 7 the number of sickling men and women and of the various types in each area are summarized.

The following electrophoretic patterns were obtained:

$$A + S \ (138), \ S + C \ (2), \ S + A + F \ (1) \text{ and } S + F \ (1).$$

The S + A + F and S + F pattern for a man from Surinam and for a woman from St. Eustatius will be commented upon later.

TABLE 7 — *The occurrence of Hb S types on the smaller Caribbean islands and in Surinam.*

	men (20 — 59 years)							women (20 — 59 years)						
	S-tests		*Hb pattern*					*S-tests*		*Hb pattern*				
	number	pos.	AS	SC	SAF	no ex.		number	pos.	AS	SC	SF	no ex.	
Anguilla	19	2	2					42	2	1			1	
St. Kitts	52	5	5					93	9	9				
Nevis	19	2	2					34	3	3				
Antigua	13	0						26	2	2				
Montserrat	26	4	4					31	1	1				
Dominica	57	5	5					55	6	5			1	
St. Lucia	78	7	7					49	8	6			2	
St. Vincent	64	8	8					72	8	7	1			
Grenada	25	1	1					24	4	3			1	
Barbados	81	5	5					21	0					
Trinidad	16	1	1					12	1				1	
St. Maarten*	129	9	8	1				62	5	5				
St. Eustatius	33	3	3					23	5	4		1		
Saba	24	3	3					14	1	1				
Aruba	27	1	1					22	0					
Bonaire	202	5	5					119	2	2				
Surinam	360	20	18		1		1	217	11	11				

* Dutch and French part of the island together.

2. THE OCCURRENCE AND DISTRIBUTION OF VARIOUS Hb S AND OTHER Hb TYPES AMONG FAMILY MEMBERS.

a. *Shell families.*

Family members were examined after the presence of Hb S in one or both parents had been established, irrespective of country of origin.

The haemoglobin of members of 222 families was examined by electrophoresis. Both parents in 16 families and one in 206 families proved to be Hb S carriers.

A summary of the total number of families, classified according to the Hb pattern of the parents shows:

	Hb S partner	other partner	number of families
Both parents Hb S carrier:	AS	AS	15
	AS	SC	1
One parent Hb S carrier:	AS	A	184
	AS	AC	13
	AS	C	1
	AS	AF	1
	SC	A	3
	SAF	A	3
	SF	A	1
			222

As many children were examined as possible. Hb patterns of children for whom the results of the serological reactions did not correspond with those of the parents were omitted when the results were worked out.

The examination was further extended to the members of 10 families in view of the Hb patterns of the parents or of the children. These 10 families from the above material, in which the Hb patterns of the parents showed respectively AS × A (5), AS × AF (1), SAF × A (3) and SF × A (1), will be dealt with separately.

All the Hb patterns AS and AC of the parents and children of the families to be dealt with here showed clearly the presence of more Hb A than Hb S or Hb C and consequently heterozygosity for the Hb S and the Hb C gene respectively.

Patterns of this sort for children whose parents have Hb patterns SC and all C, together with the haemoglobin distribution among the children, confirm the assumption that these parents were respectively doubly heterozygous for the Hb S and Hb C gene and homozygous for the Hb C gene, and also that an interacting thalassaemia gene could not be assumed. The presence of non-interacting thalassaemia and/or thalassaemia without

Hb A_2 increase cannot be proved by electrophoresis only, unless the latter were to interact with an abnormal Hb gene.

To obtain an impression of the haemoglobin distribution among the members of the sibships the families were grouped in accordance with the above-mentioned combinations of Hb patterns of the parents, classifying the families according to the number of children per family.

The following tables comprise statements of:

- the haemoglobin distribution among the members of the sibships (total, male and female children);
- the number of children not examined and the number of children deceased among these members;
- the total number of male and female children of the families.

Children who had shown morbid symptoms of sickle cell anaemia and who had died before this investigation was started (and whose parents now proved to be heterozygous for the Hb S gene) are reported separately in the table concerned. They are included as homozygotes in a corrected summary of the results for these families.

The figures in the deceased column refer solely to children over three months old and consequently represent a minimum, partly though not altogether since these figures depend on the parents' returns (polyclinical data; questioning of parents during sampling).

b. *Families ascertained through paediatric patients (Dr. C. Winkel).*

Here family members were examined when the presence of Hb S had been established in connection with morbid symptoms in one of the children of these families. The haemoglobin of members of 12 families was examined by electrophoresis. In six families the propositus had an all S pattern and both parents each an AS pattern. In the other six families the propositus had a SC pattern while one of the parents showed an AS and the other an AC pattern.

Practically all children of these families were available for examination. Their serological reactions were in accordance with those of the parents.

All the Hb patterns AS and AC of the parents and the children clearly showed the presence of more Hb A than Hb S or Hb C. None of the parents showed exclusively Hb A, proving together with the mode of haemoglobin distribution that an all S or a SC pattern among the children was not determined by the presence of an interacting thalassaemia.

It was possible to determine the haemoglobin distribution among the children after the families had been grouped according to the Hb patterns of the parents. The tables concerned have been prepared as mentioned under 2a.

TABLE 8 — *The distribution of Hb S among children, both parents known to have the Hb S trait.*

Shell series $AS \times AS$

Number of Fam.	Number of Child.	Examined Male AA AS SS	Examined Female AA AS SS	Not examined Male †	Not examined Female †	Total number examined AA AS SS	Total number in families M	Total number in families F	Clinic. sickle cell anaemia
2	3	2 1 2	1			2 2 2	5	1	
2	4	2 2	2 1 1			4 3 1	4	4	
3	5	1 1 1	6 4 1	1		7 5 2	4	11	
2	6	4 1	4 1		2(2)	8 2	5	7	?
1	7	1 1 2	1 2			2 3 2	4	3	
2	8	2 4 1	2 4 1	2(1)		4 8 2	9	7	1 ♂ †
1	9	1 1 1	5		1(1)	1 6 1	3	6	1 ♀ †
1	13	2 1	1 7	1	1(1)	3 8	4	9	?
1	13	1 2	4	6(3)		5 2	9	4	?
15	99	12 17 8	16 28 4	10(4)	4(4)	28 45 12	47	52	1 ♂ † 1 ♀ †

minus last family (13 child. 6 missing);
plus 2 † children, not examined but clinically sickle cell anaemia:

14	86	11 15 9	12 28 5	3	3(3)	23 43 14	38	48	—
Fam.	Child.	AA AS SS Male	AA AS SS Female	M.†	F.†	AA AS SS Total	M. Total	F. Total	

expected ratio 1 : 2 : 1

Remarks: a) age of three male children, not examined at date of family investigation: 25, 25 and 24 years.

b) age of three † female children, not examined, at time of death: 12 and 10 years and 8 months, cause of death according to parents: chronic illness, measles?, belly-ache respectively.

TABLE 9 — *The distribution of Hb S among children, both parents known to have the Hb S trait.*

Dr. Winkel series $AS \times AS$

Number of Fam.	Number of Child.	Examined Male AA	AS	SS	Examined Female AA	AS	SS	Not examined Male†	Not examined Female†	Total number examined AA	AS	SS	Total number in families M	F	Clinic. sickle cell anaemia
1	3	1	1				1			1	1	1	2	1	
1	5			1	1	2	1			1	2	2	1	4	
2	7	2	4	1	2	2	3			4	6	4	7	7	
1	8	2	1			2	3			2	3	3	3	5	
1	10		2		3	2	1	2(1)		3	4	1	4	6	1 ♂ †
6	40	5	8	2	6	8	9	2(1)		11	16	11	17	23	1 ♂ †
plus 1 † child, not examined but clinically sickle cell anaemia:															
6	40	5	8	3	6	8	9	1		11	16	12	17	23	—

TABLE 10 — *The distribution of Hb S and Hb C among children, one parent known to have the Hb S trait and the other the Hb C trait.*

Shell series AS × AC

Number of Fam.	Number of Child.	Examined Male AA	AC	AS	SC	Examined Female AA	AC	AS	SC	Not examined Male†	Not examined Female†	Total number examined AA	AC	AS	SC	Total number families M	F
1	2	2										2				2	
1	3			1		1			1			1		1	1	1	2
2	4	1		1		3	1		2			4	1	1	2	2	6
2	5	2	2	2		1		2	1			3	2	4	1	6	4
2	6		1	1	3	2	1	1	3			2	2	2	6	5	7
2	7	1	3	3		2	3	1	1			3	6	4	1	7	7
2	8	1	2		3	1	2	3	3	1		2	4	3	6	7	9
1	10	3	1	1	2		1	1	1			3	2	2	3	7	3
13	75	10	9	9	8	10	8	8	12	1		20	17	17	20	37	38

expected ratio 1 : 1 : 1 : 1

TABLE 11 — *The distribution of Hb S and Hb C among children, one parent known to have the Hb S trait and the other the Hb C trait.*

Dr. Winkel series *AS × AC*

Number of Fam.	Number of Child.	Examined Male AA	AC	AS	SC	Examined Female AA	AC	AS	SC	Not examined Male†	Not examined Female†	Total number examined AA	AC	AS	SC	Total number in families M	F
2	4				1	3	3		1			3	3		2	1	7
2	5		2	2	5			1					2	3	5	9	1
1	7	1	1			2	1	1	1			3	2	1	1	2	5
1	9	1	1	1		1	3		2			2	4	1	2	3	6
6	34	2	4	3	6	6	7	2	4			8	11	5	10	15	19

TABLE 12 — *The distribution of Hb S and Hb C among children, one parent known to have Hb S trait and the other to have Hb S and Hb C.*

Shell series *AS* × *SC*

Number		Examined								Not examined		Total number examined				Total number in family	
		Male				Female											
of Fam.	of Child.	AC	AS	SC	SS	AC	AS	SC	SS	Male†	Female†	AC	AS	SC	SS	M	F
1	7	1	1	1	1	1		1	1			2	1	2	2	4	3

expected ratio 1 : 1 : 1 : 1

TABLE 13 — *The distribution of Hb S and Hb C among children, one parent known to have the Hb S trait and the other Hb C only.*

Shell series *AS* × *CC*

Number of Fam.	Number of Child.	Examined Male		Total number examined	Total number in family
		AC	SC	Male	
1	3	1	2	3	3

expected ratio 1 : 1

TABLE 14 — *The distribution of Hb S and Hb C among children, one parent known to have Hb S and Hb C and the other normal haemoglobin.*

Shell series *SC* × *AA*

Number		Examined				Not examined		Total number examined		Total number in families	
		Male		Female							
of Fam.	of Child.	AC	AS	AC	AS	Male†	Female†	AC	AS	M	F
2	6	1	1	7	3			8	4	2	10
1	9	1	2	1	5			2	7	3	6
3	21	2	3	8	8			10	11	5	16

expected ratio 1 : 1

TABLE 15. *The distribution of Hb S among children, one parent known to have the Hb S trait and the other normal haemoglobin.*

Shell series $AS \times AA$

Number		Examined				Not examined		Total number examined		Total number in families	
		Male		Female							
of Fam.	of Child.	AA	AS	AA	AS	Male†	Female†	AA	AS	M	F
9	1	4	3	1	1			5	4	7	2
21	2	8	16	8	10			16	26	24	18
33	3	22	28	23	25	1		45	53	51	48
20	4	26	22	16	15	1		42	37	49	31
24	5	32	22	33	29	2	2 (2)	65	51	56	64
23	6	40	28	28	35	4 (1)	3	68	63	72	66
13	7	23	20	20	26	2 (1)		43	46	45	46
15	8	24	33	23	37	2 (1)	1	47	70	59	61
9	9	14	30	16	14	5	2	30	44	49	32
4	10	12	15	5	6	2		17	21	29	11
3	11	8	9	8	6	2		16	15	19	14
3	12	10	7	10	9			20	16	17	19
1	13	3	6	3	1			6	7	9	4
1	14	1	6	2	5			3	11	7	7
179	916	227	245	196	219	21 (3)	8 (2)	423	464	493	423

expected ratio 1 : 1

3. Interactions of the Hb S gene in 10 Caribbean families

In 10 families as complete an examination as possible was made desirable in view of the Hb patterns of the parents and children and the mode of the haemoglobin distribution among the family members.

Some examinations, in particular the haematological ones, were carried out once only. The results were obtained independently of, but at the same time as, the data concerning haemoglobin determinations.

The families are grouped according to the findings.

Thus the families Q I, Q II and R, in which the fathers had Hb S as well as Hb A and Hb F, together with families S, T and U where children show corresponding patterns, will be discussed and the results summarized.

Family V, and also families W, Y and Z will then be discussed separately.

a.

FAMILY Q I. (Curaçao; eight children)

Father Q I: both electrophoresis and chromatography revealed him to have a clear SAF pattern coupled with an increase in the A_2 fraction. In the absence of anaemia the blood picture showed moderate hypochromia. The osmotic resistance was distinctly increased.

Mother Q I: had normal haemoglobin. There was a slight anaemia, probably due to iron deficiency.

Children Q I: whilst no clear differences in haemoglobin concentration, red cell count, blood film or osmotic fragility were established, it was found that some of the children 1, 2, 4 and 6 were Hb S trait carriers and three others 3, 5 and 8 had a Hb A pattern that was coupled with an increased A_2 fraction, while two of these children also had increased Hb F.
The A_2 and F values for one child with a Hb A pattern could not be determined.

Resumé:

Father:	SAF + ↑ A_2 ; Mother: A
4 children:	AS
3 children:	A + ↑ A_2
1 child:	incompletely examined (A)

FAMILY Q II. (Curaçao; four children)

Father Q II: the findings for this man corresponded with those for his brother, father Q I: a SAF pattern with increased A_2. He had a high red cell count and slight reticulocytosis in the presence of a slight anaemia. Moderate hypochromia was present.

Mother Q II: had normal haemoglobin without increased A_2 or F. Her blood picture was characterized by the presence of many elliptocytes. She was considered to be an elliptocytosis carrier.

Children Q II: the oldest child was an Hb S trait carrier. The other children 2, 3 and 4 had an increased A_2 fraction alongside Hb A, and the two youngest had distinctly increased Hb F values; at the same time they also evinced moderate anaemia with a clearly increased red cell count.

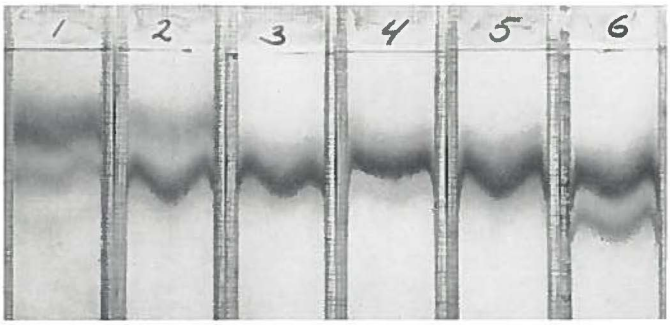

Fig. 6. Chromatographic haemoglobin patterns (fam. Q II). 1. father (S + A + F); 2. child 1 (A + S); 3, 4 & 5. child 2, 4 and 3 (A); 6. control (A + p.h. F).

The slight variations in the blood film which will be discussed in greater detail later on were not coupled with marked changes in osmotic fragility, except in child 4 (one year old, and the only child assumed to be an elliptocytosis carrier).

Resumé: Father: SAF + ↑ A_2 ; Mother: A + elliptocytosis
1 child: AS
3 children: A + ↑ A_2 (elliptocytosis in one child)

FATHER Q III: a brother of fathers Q I and Q II. Haemoglobin examination of him, his wife and his six children did not reveal any abnormalities.

MR. Q IV: an unmarried brother of fathers Q I, Q II and Q III. He was found to have an increased A_2 fraction and increased foetal haemoglobin. The blood film revealed nothing particular, besides a slight anisocytosis.
The osmotic resistance of the erythrocytes was normal. There was a relatively high red cell count compared with the haemoglobin concentration and also a slightly increased number of reticulocytes.

FAMILY R. (Surinam; four children)

Father R: the findings for this man corresponded with those for Q I and Q II from Curaçao:
distinct SAF patterns, increased A_2 and F values, together with a clearly increased osmotic resistance. The number of reticulocytes was not increased. Apart from moderate hypochromia there were remarkably few peculiarities in the blood film.

Mother R: had solely normal haemoglobin.

Children R: abnormalities were established in all the children in this family as well.
The children 1, 2 and 3 had the Hb S trait. Hb F was slightly increased in child 2. One child, 4, had normal haemoglobin with slightly increased A_2 *. There was no trace of anaemia and she was the only child that had abnormalities, albeit slight, in the blood film. The osmotic resistance was normal.

Resumé: Father: SAF + ↑ A_2 ; Mother: A
3 children: AS
1 child: A + ↑ A_2

FAMILY S. (Curaçao; thirteen children)

Father S: had an increased A_2 fraction and slight Hb F increase besides Hb A. In this case as well there was a relatively high red cell count in proportion to the slight anaemia. The abnormalities in the blood film were slight. The osmotic resistance was increased.

Mother S: the Hb S trait was established for her during electrophoretic screening. She was not available for further examination.

Children S: two children 3 and 4 had clear SAF patterns and increased A_2 fractions. Child 3 had a moderate anaemia and a relatively high red cell count. In the blood film hypochromia was quite pronounced among generally limited abnormalities. The osmotic resistance was increased.

* checked by Prof. Huisman, Augusta.

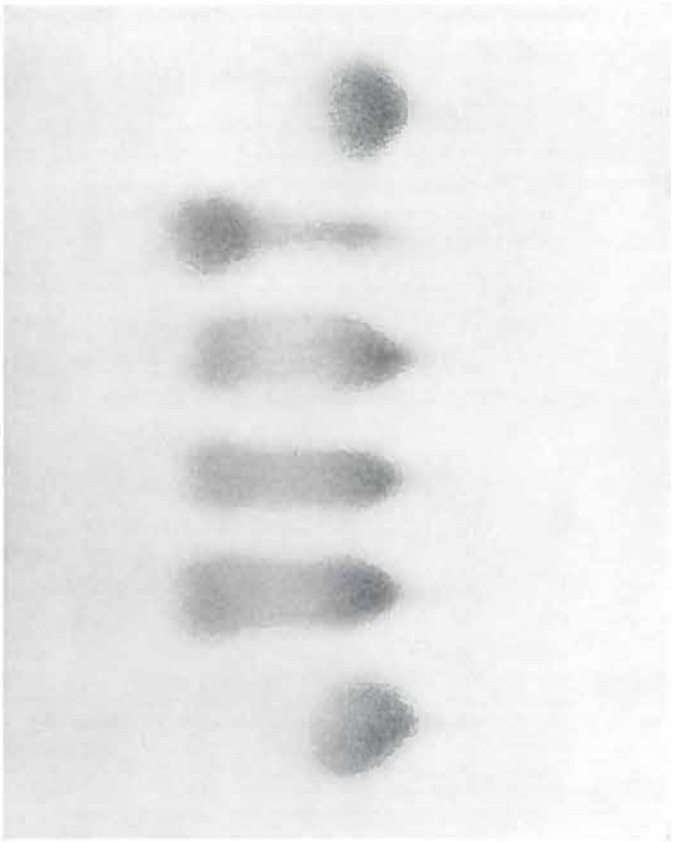

Fig. 7. Electrophoretic haemoglobin patterns (fam. R). From above: mother, father, child 1, 2, 3 and 4.

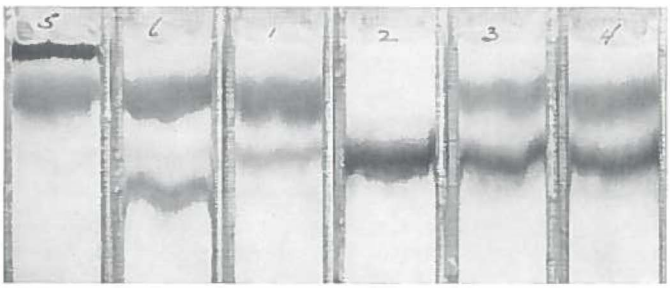

Fig. 8. Chromatographic haemoglobin patterns (fam. R and child V 2). 5. control (S + C); 6. child V 2 (S + p.h. F); 1. father R (S + A + F); 2. mother R (A); 3 & 4. child R 1 and R 2 (A + S).

The children 1, 5, 7, 10 and 13 all had the Hb S trait. It was not possible to carry out a full examination of the children 1 and 12. Child 13, aged three, had a slightly increased Hb F.
Children 6, 8, 9 and 11 were all found to have Hb A without further peculiarities.
Child 2 had besides Hb A both increased A_2 and increased F. The blood picture was normal and the osmotic fragility was not clearly reduced.

Resumé:

Father:	A + ↑ A_2 ;	Mother: AS
2 children:	SAF + ↑ A_2	
5 children:	AS	(child 1 included)
1 child:	A + ↑ A_2	
4 children:	A	
1 child:	incompletely examined (child 12)	

FAMILY T. (Curaçao; seven children)

Father T: besides Hb A this man had one of the highest A_2 values established. There was no anaemia and only very slight morphological abnormalities of the erythrocytes. The osmotic fragility was not reduced.

Mother T: had the Hb S trait without any other peculiarities.

Children T: children 3 and 4. The first of these had no anaemia and the second was slightly anaemic; both showed clear SAF patterns together with increased A_2, had an increased number of reticulocytes and only slight morphological abnormalities of the erythrocytes. The osmotic resistance was increased in both.
Child 1, not being anaemic, had like his father a high increased A_2 value and slightly increased Hb F. The blood film showed remarkably few peculiarities. The osmotic fragility was not noticeably changed.
It was not possible to examine the two oldest children.

Resumé:

Father:	A + ↑ A_2 ;	Mother: AS
2 children:	SAF + ↑ A_2	
1 child:	A + ↑ A_2	
2 children:	A	
2 children:	not examined.	

FAMILY U. (Curaçao; five children)

Father U: an Hb S trait carrier; examination revealed nothing of particular interest.

Mother U: as well as a very slight Hb F increase there was a clear A_2 increase. She had a moderate anaemia and her red cell count was high; her blood film showed a rather distinct anisocytosis and ovalocytes occurred. The osmotic fragility was not clearly altered.

Children U: child 4, aged six, had an SF pattern in which, after more than one test, it was not possible to "separate" the A_2 fraction from the excess of Hb S. (The value obtained is reported for the sake of completeness and should be accepted accordingly as such). This boy had a definite anaemia with a low haemoglobin con-

centration and a low red cell count and reticulocytosis. The osmotic resistance was clearly increased. The significant blood picture will be discussed in detail later on.
The children 2 and 5 had besides Hb A increased A_2 values, whilst the first of these had an increased Hb F value and a high red cell count with a moderate anaemia. The blood pictures corresponded with that of the mother. The osmotic resistance of the erythrocytes was increased in both children.
Child 1 had normal Hb A and child 3 the Hb S trait.

Resumé:

Father:	AS	; Mother: A + ↑ A_2
1 child:	SF	
2 children:	A + ↑ A_2	
1 child:	AS	
1 child:	A	

Before a conclusion is reached regarding the findings in the above families, the blood pictures of the persons with normal haemoglobin and increased A_2 on the one hand and those of persons with SAF and SF patterns on the other should be discussed.

The blood pictures.

The blood pictures of mother U and two of her children correspond with those of the "classic" thalassaemia trait:

- besides anisocytosis and poikilocytosis the moderate hypochromia is the first to strike one. The blood picture is further characterized by oval cells which are clearly elliptic to rod-shaped (fig. 9). A single target cell and a somewhat intensified punctate basophilia are also observed.

The blood pictures of the persons with normal haemoglobin plus increased A_2 in the other families (Q I and Q II, together with R, S and T) differ markedly from those above and are therefore termed "non-classic" (fig. 10):

- the hypochromia is not marked, being just indicated and no more. The erythrocytes show a slight anisocytosis. Some oval cells, whose shape is best described as "oval" (not clearly elliptic and never rod-shaped) are met with.

  In most films target cells occur now and then. The abnormalities are, however, so slight that the blood film is not immediately spotted as abnormal.

  These are therefore pictures which can also be found in normal persons, and no conclusions may accordingly be drawn from the film alone.

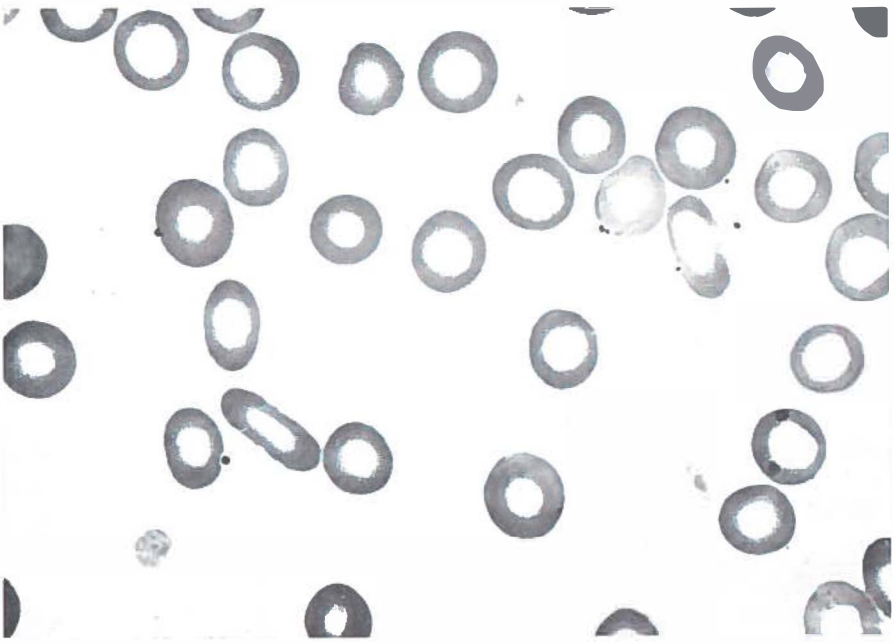

Fig. 9. "Classic" trait (child U 2).

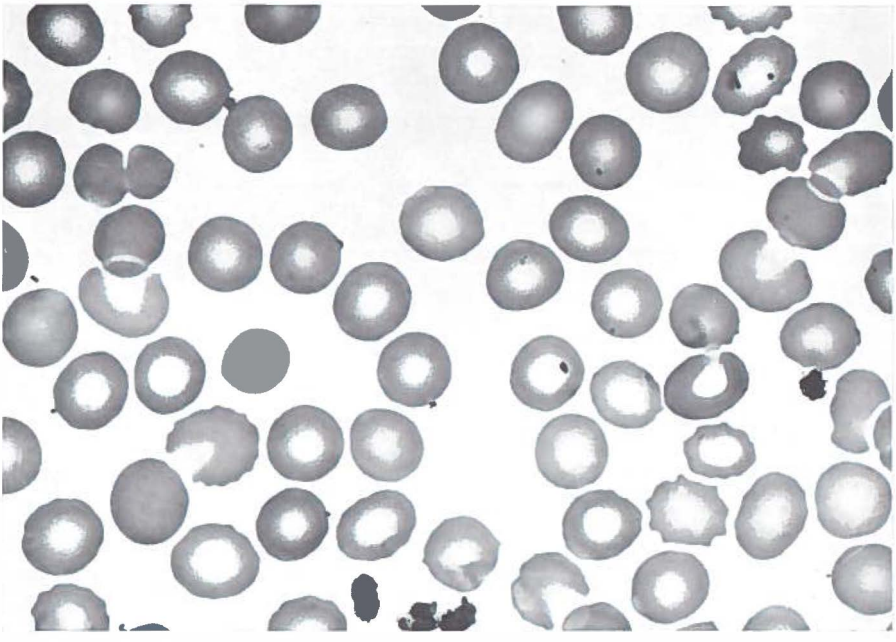

Fig. 10. "Non-classic" trait (child Q II 2).

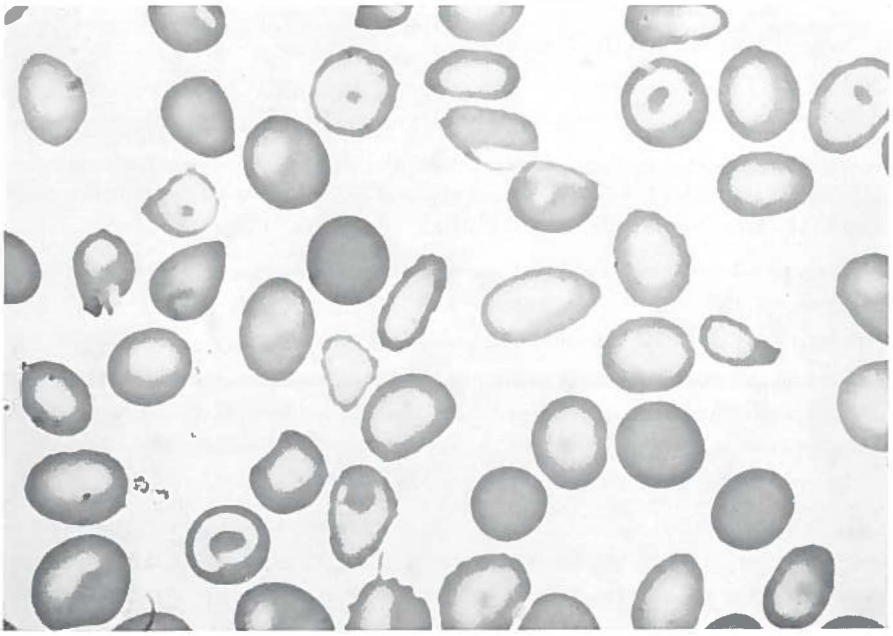

Fig. 11. "Classic" sickle cell-thalassaemia (child U -l).

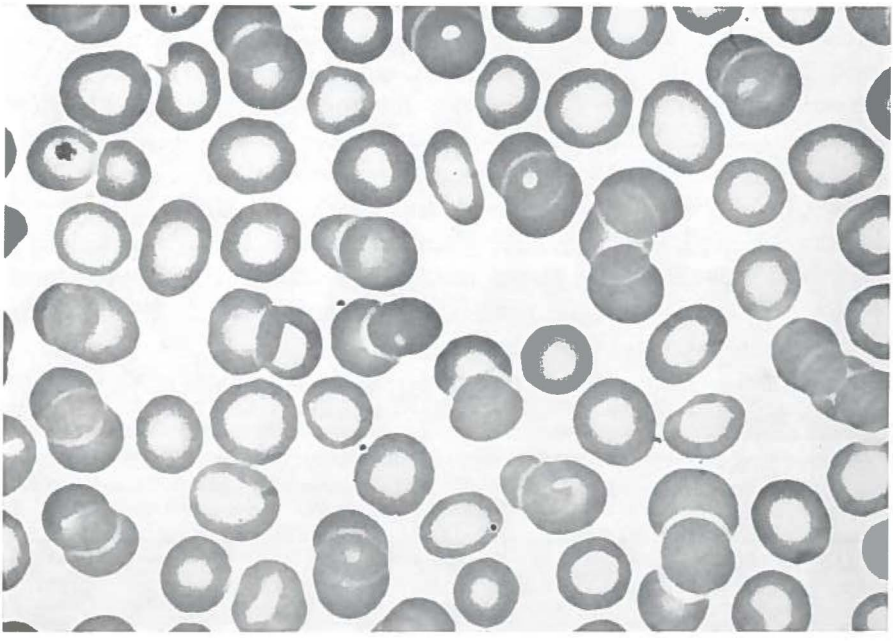

Fig. 12. "Non-classic" sickle cell-thalassaemia (father R).

The blood picture of child U 4 (fig. 11) corresponds with those of the "classic" sickle cell-thalassaemia:

– there is a very irregular picture of large and small erythrocytes which are badly filled with haemoglobin. Target cells are immediately apparent. There is a marked polychromasia. Many erythrocytes are elliptic or pear-shaped or completely irregular. No clear sickle cells were seen in the film. Some cells show distinct basophilic stippling.

The blood picture observed in the parents and children with SAF patterns in the families Q I and Q II, together with R, S and T, and which is here termed "non-classic", showed (fig. 12):

– a moderate hypochromia which is not, however, so marked whilst the morphological picture is only very slightly irregular. Although anisocytosis is present most of the erythrocytes are round. Polychromasia is only slight and basophilic stippling is not distinct.

In family U the slight anaemia, the increased Hb A_2 and the typical morphological picture of the mother and some of the children on the one hand and the marked anaemia, the SF pattern and an equally typical morphological cell picture of one of the children on the other, show that the "classic" thalassaemia trait is present and has led to interaction with the Hb S gene.

In the other families Q I, Q II, R, S and T, increased A_2 either with or without increased Hb F was observed in various children and parents, while others also had changed haemoglobin ratios which resulted in SAF patterns. These families are, however, distinguished from family U in that the trait carriers and the heterozygotes for both this trait and the Hb S gene were not clearly anaemic. Furthermore morphological pictures barely differing from the normal were observed in the single trait carriers. The blood pictures of the "non-classic" trait, in which there is no hypochromia or cell irregularity, are "characterized" by the almost total absence of abnormalities; it is practically impossible to distinguish them from the pictures of normal persons, and they are accordingly markedly different from those of the "classic" trait.

The different traits were found to occur as shown below in the children of these families:

TABLE 16 – *The distribution of the Hb S trait and the "non-classic" and "classic" thalassaemia trait among Curaçao children, one parent known to have the Hb S trait and the other the "non-classic" or "classic" thalassaemia trait.*

Type	Fam.	S trait	Thal. trait	S thal.	Normal	Not exam.	Total
non-classic	S & T	5	2	4	6	3	20
classic	U	1	2	1	1	–	5

Study of the haemoglobin distribution among the children from three of these families (Q I, Q II and R), where the father was heterozygote for both the "non-classic" trait and for the Hb S gene, reveals the following:

TABLE 17 – *Types of children resulting from union of normal individuals with persons with "non-classic" sickle cell-thalassaemia.*

Family	Origin	S trait	Non-classic trait	Not exam.	Total
Q I	Curaçao	4	3	1	8
Q II	Curaçao	1	3	–	4
R	Surinam	3	1	–	4
Q I + Q II + R		8	7	1	16

Conclusions.

The results of the examination of these six families show that:

- the "classic" thalassaemia trait occurs on Curaçao;
- a "non-classic" trait quite distinct from the above also occurs both on Curaçao and in Surinam;
- the gene responsible for this trait is allelomorphic or closely linked with the Hb S gene.

The "non-classic" trait was encountered more frequently than the "classic" trait in this material.

It appears likely that this would be the case in general, but a conclusion cannot be drawn without further evidence.

b.

FAMILY V. (Nevis; two children)

Father V: in this non-anaemic man 32% Hb F was present alongside Hb A, whilst the A_2 value was low. The blood picture was normal. The osmotic resistance of the erythrocytes was increased.

Mother V: a woman with anaemia and a low serum iron value and who appeared to have the Hb S trait. Target cells, anisocytosis and hypochromia were found in her blood film.

Children V: child 2 had an SF haemoglobin pattern and a low A_2 value. The proportion of Hb F was 38%. There were many target cells present in the blood picture; sickle cells were not observed. The osmotic resistance was increased.
Child 1 had a large amount of Hb F alongside Hb A just like her father. The A_2 value was low. Her blood film revealed nothing of special interest. The osmotic resistance was increased.

Resumé:	Father:	AF + ↓ A_2 ;	Mother:	AS
	1 child:	SF + ↓ A_2		
	1 child:	AF + ↓ A_2		

The findings for the father and two children, i.e. the high Hb F values, the low normal Hb A_2 values and the increased osmotic resistance of the erythrocytes indicate that persistent high foetal haemoglobin is present in this family and that the SF haemoglobin pattern of one of these children depends on heterozygosity for the Hb S gene as well as the persistent high Hb F anomaly.

c.

FAMILY W. (St. Maarten; two children)

Father W:	had normal haemoglobin. The Hb F and Hb A_2 values were not increased. The blood picture was normal.
Mother W:	was a Hb S trait carrier. Her blood picture did not show any peculiarities.
Children W:	Child 1 was examined after being operated on at the age of one (splenectomy) for hypersplenism of unknown origin. Increased osmotic resistance of the erythrocytes had twice been established before the operation. This child was found to have a SF haemoglobin pattern. Foetal haemoglobin amounted to 33 %. The A_2 fraction was normal. His blood picture was characterized by marked polychromasia, anisocytosis and the presence of target cells, sickle cells and normoblasts. Moderate hypochromia was present. Child 2, aged six months, was a Hb S trait carrier and had 6.6% Hb F. His blood picture showed anisocytosis and hypochromia.

Resumé	Father:	A ;	Mother:	AS
	1 child:	SF		
	1 child:	AS		

It was not possible to determine the serum iron when this family was examined.

The SF pattern of child 1 was obtained after splenectomy. The increased osmotic resistance found twice before the operation could well have been influenced by unknown complicating factors.

Even if these factors could be ignored, the findings in this family do not permit definite conclusions. The fact that the osmotic resistance was increased in both parents and child 2 leads one to doubt the accuracy of these osmotic fragility observations.

Of the three possible ways of explaining the SF haemoglobin pattern of child 1, different paternity should be assumed. Previous examinations had revealed target cells in the blood film of the father, but it was not possible to confirm the presence of target cells during the present examination.

Homozygosity for the Hb S gene seems unlikely as the Hb F content was higher than would be expected in that case. The anaemia and the course of the illness when one year old both make heterozygosity for the Hb S gene and the persistent high Hb F anomaly improbable. The Hb A_2 value was not found to be low normal either.

The fact that increased osmotic resistance of the erythrocytes was found in this child twice before and once after the operation could be an indication of double heterozygosity for the Hb S and a thalassaemia gene.

In order to explain the SF pattern in this way one must either 1) assume different paternity, or 2) presume an "interacting" thalassaemia not resulting in an increase of Hb A_2 or in morphological abnormalities of the erythrocytes in the father.

FAMILY Y. (St. Eustatius; four children)

Father Y:	had normal haemoglobin and normal Hb F and A_2 values. His blood picture showed no peculiarities. The osmotic resistance was normal.
Mother Y:	haemoglobin examination revealed a SF pattern with a normal quantity of Hb A_2; the Hb F content was 29%. There was a slight anaemia. A marked anisocytosis together with poikilocytosis and hypochromia was observed in the blood picture, whilst oval cells were found regularly distributed in the film, but no sickle cells. The osmotic resistance was moderately increased.
Children Y:	all four children had a Hb S trait pattern and normal A_2 values. In the children 2, 3 and 4 (the youngest of which was four years old), increases in the foetal haemoglobin of respectively 7.3, 4.6 and 7.0% were established. Target cells were found to be regularly distributed in the blood pictures of these three children. The youngest child also had anisocytosis and mild hypochromia, and the osmotic resistance was increased in his case.

Resumé:

Father:	A ; Mother: SF + normal A_2 (F = 29%)
3 children:	AS + ↑ F + normal A_2
1 child:	AS

All four children of this family had the Hb S trait, whilst for three of them an increase in the Hb F and a rise in the number of target cells above that observed in the Hb S trait was established. In view of the entirely normal pattern and picture of the father, it is probable that the factor determining these increases is derived from the mother, as is the Hb S. If the children have inherited this factor from the mother, it must be assumed that the Hb S gene and this X gene are not alleles. Furthermore, the mother must be heterozygous for this gene since one of the children evidently does not possess this factor.

If it is assumed that the mother is heterozygous for the Hb S gene, a

picture corresponding with that for three of her children could be expected. This is not, however, the case.

The fact that no Hb A was found in her supports the possibility that this woman might be homozygous for the Hb S gene. If that is the case it must be concluded that the "abnormal" *X* gene has led to an extremely high Hb F content.

If it were to be assumed that the abnormal *X* gene is derived from the father, although evidently non-penetrant here, the distinctly atypical picture of the mother cannot be explained unless one assumes that she possesses a further hereditary factor which she has not transmitted to her children.

The most probable explanation is that this woman is homozygous for the Hb S gene and heterozygous for the abnormal *X* gene, and this seems apparently to be accompanied by a virtually asymptomatic carrier state (see further).

This assumption could have been confirmed by examination of the woman's parents, but this was not, however, possible. It is probable that both her parents had a Hb S gene and one of them the abnormal *X* gene. This doubly heterozygous grandparent should then have a picture similar to those found in three of the grandchildren. It is likely that it will be possible to study the heterozygous state of the abnormal *X* gene in the grandchildren of mother Y.

FAMILY Z. (St. Vincent; two children)

Only haemoglobin examinations could be carried out in this family.

Father Z:	had Hb A and 1% Hb F together with 2.9% Hb A_2.
Mother Z:	was a Hb S trait carrier; had 1% Hb F and 2.7% Hb A_2.
Children Z:	The younger child (15 years old) showed an SAF pattern in which the Hb F content was 6.7% and the Hb A_2 content 8.7%. On one occasion a haemoglobin concentration of 10.5 gr% together with a red cell count of 4.1 mill. cm^3 was established. Four months later the haemoglobin concentration was 12.5 gr.%. The blood picture was never assessed. The other child (18 years old) was a Hb S trait carrier and had 1% Hb F and 3.5% Hb A_2.

The sole conclusion that could be reached from this examination was based on the haemoglobin pattern and the high A_2 value, and was that the younger child was heterozygous for both the Hb S gene and a thalassaemia gene.

FAMILY—TABLES

TABLE 18 — *Family Q 1.*

Q 1 Curaçao	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C. (mill. cm³)	P.C.V.	M.C.V.	M.C.H.C	M.C.H.
Fa Q1		O +	+	SAF	SAF	7.6	5.0	14.1	4.5	42	93	34	31
Mo Q1		O +	—	A	A	nil	2.2	12.7	5.2	41	79	31	25
Q1 1	f 15	O +	+	AS	AS	1.5	2.4	14.1	4.6	46	100	31	31
Q1 2	m 13	O +	+	AS	AS	1	2.3	12.4	4.4	46	106	27	28
Q1 3	m 11	O +	—	A	A	1	6.0	12.1	4.5	40	89	30	27
Q1 4	f 9	O +	+	AS	AS	<1.5	2.3	12.2	4.8	38	80	32	26
Q1 5	f 8	O +	—	A	A	5.0	6.0	11.8	4.9	40	82	30	24
Q1 6	f 6	O +	+	AS	AS	<1	3.2	11.7	4.5	38	85	31	26
Q1 7	f 4½	O +	—	A	—	—	—	12.5	3.9	35	89	36	32
Q1 8	m 1¼	O +	—	A	A	6.3	4.9	10.5	4.2	32	76	33	25

results				Osmotic fragility						Serum iron values		
(%)	Ind. bil.	W.B.C.	Bloodfilm	0.50 % 0–5 %	0.45 % 5–45 %	0.40 % 50–90 %	0.35 % 90–99 %	0.30 % 97–100 %	0.20 %	Serum iron (μg %)	Total capacity	Saturation (%)
4	0.3	6400	hypochromia ++ anisocytosis ++ poikilocytosis + basophilic stipp ±	0	4	17	61	92	100	117	351	33
5	0.3	6600	anisocytosis	12	?	40	78	94	100	71	381	19
1	0.2	5400	normal	—	9	53	96	99	100	81	342	24
1	0.3	10300	anisocytosis	2	8	37	81	98	100	148	402	37
3	0.2	7500	no clear abnormalities	4	25	73	98	98	100	27	423	6
5	0.2	6700	normal	6	16	54	88	95	100	114	393	29
4	0.2	6100	anisocytosis	—	6	43	83	92	100	134	402	33
3	0.2	7700	anisocytosis	3	10	42	89	95	100	61	393	16
2	—	12400	no clear abnormalities	—	—	—	—	—	—	—	—	—
3	0.3	10500	anisocytosis hypochromia	?	?	75	94	98	—	96	393	24

TABLE 19 — *Family Q II.*

Q II Curacao	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa QII		O +	+	SAF	SAF	3.0	5.2	12.5	3.9	40	68	31	21
Mo QII		B +	—	A	A	<1	2.9	13.2	4.9	40	82	33	27
QII 1	f	7½ B +	+	AS	AS	<1	2.2	12.2	4.6	37	80	33	27
QII 2	m	6 O +	—	A	A	1	4.4	10.3	5.0	36	72	29	21
QII 3	m	3½ O +	—	A	A	4.6	4.5	10.9	5.6	40	72	27	20
QII 4	f	11/6 B +	—	A	A	9.5	3.9	11.6	4.8	38	79	31	24
Fa. QIII		A +	—	A	A	1.5	2.2	15.4	5.2	45	86	34	29
Mr QIV		O +	—	A	A	4.0	5.6	13.8	5.3	36	68	38	26

results				Osmotic fragility						Serum iron values		
Reticulocytes (‰)	Ind. bil.	W.B.C.	Bloodfilm	0.50 %	0.45 %	0.40 %	0.35 %	0.30 %	0.20 %	Serum iron (µg %)	Total capacity	Saturation (%)
				0–5 %	5–45 %	50–90 %	90–99 %	97–100 %				
21	0.5	5700	hypochromia ++ anisocytosis ++ poikilocytosis + polychromasia + ovalocytosis + target cells + basophilic stipp.	1	4	8	42	78	97	116	273	42
9	0.3	5100	ovalocytosis +++ no hypochromia	7	34	83	94	97	—	151	351	43
3	0.3	3700	normal	6	23	73	93	100	100	76	372	20
10	0.1	7400	anisocytosis + hypochromia + ovalocytosis +	3	14	61	85	98	100	142	372	38
7	0.2	6100	hypochromia ± ovalocytosis ± anisocytosis	2	6	42	89	95	100	—	—	—
16	0.4	9900	anisocytosis ++ poikilocytosis ++ ovalocytosis ++ hypochromia	0	1	5	12	99	100	93	342	27
3	0.7	6600	normal	4	19	70	78	92	100	140	393	36
20	0.6	4000	anisocytosis	12	16	33	71	96	100	147	426	35

TABLE 20 — *Family R.*

R Surinam	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa	R	O +	+	SAF	SAF	4.3	5.1	13.5	4.8	47	96	29	28
Mo	R	B +	—	A	A	<1	2.9	13.2	4.5	43	95	31	29
R 1	m 12	B +	+	AS	AS	<1	3.1	15.9	5.1	55	107	29	31
R 2	f 10½	B +	+	AS	AS	2.2	2.1	16.0	4.8	46	95	35	33
R 3	f 9	B +	+	AS	AS	1.5	3.0	14.1	4.5	44	97	32	31
R 4	f 7½	B +	—	A	A	10.0	3.9	15.1	4.9	41	84	37	31

results				Osmotic fragility						Serum iron values		
(‰)	Ind. bil.	W.B.C.	Bloodfilm	0.50 %	0.45 %	0.40 %	0.35 %	0.30 %	0.20 %	Serum iron (μg %)	Total capacity	Saturation (%)
				0–5 %	5–45 %	50–90 %	90–99 %	97–100 %				
	0.6	5950	anisocytosis ++ hypochromia ++ ovalocytosis +	0	0	1	10	36	87	105	363	29
	0.3	5500	normal	2	25	89	91	97	98	81	327	25
	0.5	7100	normal	0	2	11	82	95	100	98	303	32
	0.6	7800	normal	1	5	65	83	95	95	—	—	—
	0.3	7000	normal	1	5	35	75	100	100	150	402	37
	0.4	7600	ovalocytosis ++ poikilocytosis + anisocytosis +	11	22	30	78	92	100	134	411	33

TABLE 21 — *Family S.*

S Curaçao	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa S		AB	+ —	A	A	2.7	5.2	12.9	4.8	36	75	36	27
Mo S		O	+ +	AS	AS	—	—	—	—	—	—	—	—
S 1	f 22	A	+ +	AS	AS	—	—	—	—	—	—	—	—
S 2	m 21	A	+ —	A	A	6.5	6.5	13.5	4.6	46	100	29	29
S 3	f 20	B	+ +	SAF	SAF	11.6	5.5	11.4	4.3	38	88	30	26
S 4	m 18½	B	+ +	SAF	SAF	11.0	6.0	14.2	5.4	46	85	31	26
S 5	m 16	A	+ +	AS	AS	<1	2.9	13.5	4.5	39	86	35	30
S 6	f 15	B	+ —	A	A	<1	2.7	12.9	3.9	41	106	31	33
S 7	m 13	B	+ +	AS	AS	<1	2.8	14.4	5.2	45	87	32	28
S 8	f 11½	B	+ —	A	A	<1	2.3	11.7	4.4	33	75	35	26
S 9	m 7½	B	+ —	A	A	1	1.9	11.6	4.8	32	67	36	24
S 10	f 6½	A	+ +	AS	AS	1.4	2.4	11.7	4.2	40	95	29	28
S 11	f 5½	O	+ —	A	A	1.5	2.1	12.8	4.2	42	99	31	30
S 12	m 4	—	— —	—	—	—	—	11.7	3.9	41	106	29	30
S 13	f 3	A	+ +	AS	AS	3.4	1.8	10.8	4.0	36	91	30	27

results				Osmotic fragility						Serum iron values		
[illegible] (%)	Ind. bil.	W.B.C.	Bloodfilm	0.50 % 0–5 %	0.45 % 5–45 %	0.40 % 50–90 %	0.35 % 90–99 %	0.30 % 97–100 %	0.20 %	Serum iron (μg %)	Total capacity	Saturation %
5	0.1	3100	anisocytosis + ovalocytosis + target cells +	0	1	14	54	88	100	111	363	31
—	—	—		—	—	—	—	—	—	—	—	—
—	—	—		—	—	—	—	—	—	—	—	—
14	0.6	8100	normal	—	5	30	75	100	100	59	363	16
11	0.3	3700	hypochromia +++ anisocytosis + ovalocytosis + target cells +	—	3	4	32	99	100	111	393	28
7	0.4	4400	hypochromia ++ anisocytosis + ovalocytosis + target cells +	—	2	6	21	61	95	134	303	41
3	0.3	2800	target cells spor.	2	5	30	91	95	100	80	324	25
7	0.8	4600	normal	2	17	52	92	93	100	128	372	34
7	0.3	4200	normal	—	3	24	82	86	100	89	294	30
5	0.2	4200	normal	2	22	85	97	99	100	88	402	22
4	0.3	6200	normal	1	5	41	91	97	98	150	372	40
7	0.3	10100	normal	—	11	50	81	94	100	56	273	21
12	0.5	8000	normal	7	20	68	88	89	100	64	—	—
7	—	5100	normal	—	—	—	—	—	—	—	—	—
5	0.2	11200	normal	3	14	34	71	90	100	—	—	—

TABLE 22 – *Family T.*

T Curaçao	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa T		B	+	A	A	1.7	8.0	14.6	4.9	46	94	32	30
Mo T		A	+	AS	AS	1	3.4	12.4	4.3	40	93	31	29
T 1	m 23	B	+	A	A	2.5	8.1	14.6	5.4	50	93	29	27
T 2	m 22	B	+	A	A	<1	3.1	15.1	5.1	46	90	33	30
T 3	m 19½	A	+	SAF	SAF	9.5	6.3	14.7	5.2	40	77	37	28
T 4	m 17½	O	+	SAF	SAF	14.3	6.1	12.7	4.8	38	79	33	26
T 5	m 15½	B	+	A	A	1	3.3	14.3	5.0	43	86	33	29

(two children not examined)

results				Osmotic fragility						Serum iron values		
(%)	Ind. bil.	W.B.C.	Bloodfilm	0.50 %	0.45 %	0.40 %	0.35 %	0.30 %	0.20 %	Serum iron (μ g %)	Total capacity	Saturation (%)
				0–5 %	5–45 %	50–90 %	90–99 %	97–100 %				
2	0.4	2800	anisocytosis + ovalocytosis + basophilic st. ±	1	8	48	81	96	100	66	324	20
4	0.1	4800	normal	1	7	48	84	94	94	113	372	31
2	0.2	4600	anisocytosis + ovalocytosis +	—	4	20	92	100	100	137	372	37
5	0.4	3700	normal	—	2	51	91	95	100	157	384	41
22	0.5	4200	hypochromia ++ anisocytosis + polychromasia target cells	—	—	9	35	78	97	104	273	38
9	0.5	4300	anisocytosis ++ hypochromia + poikilocytosis + target cells + ovalocytosis + polychromasia	—	—	9	43	88	100	136	363	37
1	0.1	4900	normal	—	6	61	93	98	100	109	423	26

TABLE 23 – *Family U*

U Curaçao	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa U		O +	+	AS	AS	<1	2.3	15.0	5.1	45	88	33	29
Mo U		B +	—	A	A	1.9	6.4	11.0	5.1	38	74	29	21
U 1	m 12	O (neg.cde)	—	A	A	<1	2.8	14.3	4.5	49	109	29	32
U 2	f 10	B +	—	A	A	3.1	5.9	10.7	5.1	40	79	27	21
U 3	m 8	O +	+	AS	AS	nil	2.6	13.5	4.9	48	97	28	27
U 4	m 6	B +	+	SF	SF	28.0	(3.1)	8.3	3.3	28	85	30	25
U 5	m 3	O +	—	A	A	<1	6.5	12.1	4.7	40	85	30	26

results				Osmotic fragility						Serum iron values		
(‰)	Ind. bil.	W.B.C.	Bloodfilm	0.50 % 0–5 %	0.45 % 5–45 %	0.40 % 50–90 %	0.35 % 90–99 %	0.30 % 97–100 %	0.20 %	Serum iron (μg %)	Total capacity	Saturation (%)
2	0.2	9700	normal	2	7	53	91	99	100	174	384	45
3	0.2	6800	anisocytosis ++ hypochromia ++ ovalocytosis + polychromasia + target cells spor. poikilocytosis ±	3	10	39	67	100	100	79	378	21
5	0.4	12400	normal	10	48	92	100	100	100	101	324	31
3	0.2	9900	hypochromia ++ ovalocytosis ++ anisocytosis + target cells spor.	2	7	30	61	91	100	68	381	18
1	0.2	9700	normal	9	13	57	72	83	100	96	411	23
2	0.6	8400	anisocytosis +++ hypochromia +++ poikilocytosis ++ target cells ++ polychromasia ++ sickle cells ?	—	—	5	35	52	97	104	384	27
2	0.3	13500	anisocytosis + hypochromia + ovalocytosis + target cells spor.	—	3	26	71	94	100	74	450	16

TABLE 24 — *Family V.*

V Nevis	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C	M.C.H.
Fa V		O +	—	AF	AF	32.0	1.0	15.4	5.1	53	105	29	31
Mo V		O +	+	AS	AS	1.4	2.6	9.6	3.6	27	76	36	27
V 1	f 6½	O +	—	AF	AF	38.0	1.0	11.8	3.8	33	86	36	31
V 2	m 5½	O +	+	SF	SF	38.0	1.6	12.6	4.7	42	89	30	27

TABLE 25 — *Family W.*

W St. Maarten	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa W		B +	—	A	A	<1	2.7	13.0	5.0	41	82	32	26
Mo W		O (neg.cde)	+	AS	AS	1.5	3.3	12.4	3.8	38	100	33	33
W 1	m 2	O +	+	SF	SF	33.0	2.5	8.5	2.5	25	99	34	34
W 2	m ½	B (neg.cde)	—	AS	AS	6.6	2.1	9.9	4.5	32	71	31	22

results				Osmotic fragility						Serum iron values		
Ret-cytes (‰)	Ind. bil.	W.B.C.	Bloodfilm	0.50 %	0.45 %	0.40 %	0.35 %	0.30 %	0.20 %	Serum iron (µg %)	Total capacity	Saturation (%)
				0–5 %	5–45 %	50–90 %	90–99 %	97–100 %				
10	0.8	3800	normal	—	1	21	69	88	98	124	396	31
7	0.3	6200	target cells + anisocytosis hypochromia	—	1	7	42	76	100	31	372	8
6	0.5	6600	normal	—	4	25	78	94	100	131	327	40
12	0.2	8200	target cells +++ anisocytosis no sickle cells	—	—	1	10	60	96	108	279	39

results				Osmotic fragility						Serum iron values		
Ret-cytes (‰)	Ind. bil.	W.B.C.	Bloodfilm	0.50 %	0.45 %	0.40 %	0.35 %	0.30 %	0.20 %	Serum iron (µg %)	Total capacity	Saturation (%)
				0–5 %	5–45 %	50–90 %	90–99 %	97–100 %				
8	0.8	4100	normal	—	10	17	63	95	100	—	—	—
5	0.5	4400	normal	1	1	8	64	99	99	—	—	—
112	0.6	17900	polychromasia +++ anisocytosis ++ target cells ++ sickle cells ++ normoblasts + hypochromia +	2	3	8	20	60	91	—	—	—
5	0.6	8250	anisocytosis + hypochromia +	1	1	4	23	74	97	—	—	—

TABLE 26 — *Family Y.*

Y St. Eustat.	Sex & age in years	Bloodgroup	S test	Phys. chem. results: Pap. electr.	Chromat.	F %	A_2 %	Haematological: Hb (gr %)	R.B.C.	P.C.V.	M.C.V.	M.C.H.C.	M.C.H.
Fa Y		O (neg.cde)	—	A	A	<1	2.5	14.0	5.1	43	84	33	27
Mo Y		O	+	SF	SF	29.0	2.0	11.4	3.3	33	99	35	34
Y 1	m 10	O	+	AS	AS	1	2.6	13.4	5.7	43	75	31	23
Y 2	m 8½	O	+	AS	AS	7.3	2.8	11.3	3.8	40	104	28	29
Y 3	f 6	O	+	AS	AS	4.6	3.2	11.8	4.5	37	83	32	26
Y 4	m 4	O	+	AS	AS	7.0	2.1	12.5	5.2	38	73	33	24

results				Osmotic fragility						Serum iron values		
(g%)	Ind. bil.	W.B.C.	Bloodfilm	0.50 % 0–5 %	0.45 % 5–45 %	0.40 % 50–90 %	0.35 % 90–99 %	0.30 % 97–100 %	0.20 %	Serum iron (μg %)	Total capacity	Saturation (%)
7	0.3	5200	normal	2	15	77	94	97	100	88	303	29
8	0.3	5300	anisocytosis +++ hypochromia ++ poikilocytosis ++ polychromasia ++ ovalocytosis + target cells +	3	8	33	77	93	100	34	300	11
7	0.4	6750	normal	5	34	77	93	99	100	47	477	10
8	0.2	3800	target cells	3	11	67	92	98	100	96	342	28
4	0.2	8000	target cells	2	3	18	78	92	99	93	402	23
8	0.2	6200	anisocytosis + hypochromia + target cells +	—	2	8	37	83	98	152	273	55

4. Observations on the fitness of Hb S carriers.

As polyclinical and clinical records were available – the results of many years' work by my colleagues in Shell Curaçao's medical service – it was possible to form an idea of the fitness of the Hb S carriers examined.

The material was suitable for a brief comparative study of the children of 0–15 years of age with sickle cell anaemia on one hand and SC haemoglobinopathy on the other and whose ailments could be checked over five-year periods.

The frequency of ailments in a small number of adult Hb SC carriers was recorded.

It was possible to check whether in the course of the years workers with the Hb S trait were found to have abnormalities which could be connected with this trait. The frequency and nature of ailments in persons with "non-classic" sickle cell-thalassaemia has been added, together with corresponding data for persons with a Hb SF pattern.

As regards the frequency of ailments the first visit to the polyclinic only has been recorded and not the number of "follow-ups", which depended to some extent on the doctor in charge of the case. The "hospitalization frequency" refers solely to periods spent in hospital as a result of the carrier's haemoglobin.

The Hb S homozygous and Hb SC heterozygous children. The greatest number of complaints in Hb S homozygous children occurred between the ages of 3 and 5. Two of the fourteen children died before they were 4 yrs old. The anaemic children usually appeared at the polyclinic with fevers and gastro-intestinal troubles and much pain in the extremities. One three-year old boy, for whom high Hb F values were established, was an exception; he was certainly anaemic, but had no pains or ailments of a more general nature.

General complaints were found to be less common in children from 5–10 yrs old whilst abdominal pains occurred more frequently.

Some children between 10–15 yrs of age were found to have no ailments connected with the haemoglobinopathy, whilst other had more complaints than when they were younger. All children had an anaemia of 5–10 gr%.

The highest age at which the spleen was palpable in these children was 11 yrs. The liver was practically always enlarged.

None of the 23* double-heterozygous Hb SC children died. The ailments

* It was possible to add three children, who were not involved in the family investigation,

connected with their haemoglobinopathy occurred sporadically – with one exception – and were usually not of a general nature.

These complaints were not found to occur more frequently in certain age groups.

For some children from 0–5 yrs old medical advice was only requested on one occasion, as a result of jaundice. The children who came to the polyclinic more frequently suffered from pains, mainly in the extremities. There were also children without ailments in all groups. The haemoglobin content of the children with SC haemoglobinopathy varied from 7–12 gr%, being usually 9–11 gr%. The spleen was also felt in 15-year old children. A splenectomy was carried out on one ten-year old girl because of hypersplenism and extreme painfulness of the greatly enlarged spleen. In children 0–10 yrs old this organ and the liver was as often as not palpable.

When these two groups of children are compared it can be seen that the number of ailments and diseases is appreciably greater for those with sickle cell anaemia than for the double-heterozygous Hb SC children. The former have chronic complaints of a more general nature during the earlier years and suffer from recurrent pains. They are children "in distress", as opposed to the double-heterozygous Hb SC children, who may have incidental complaints of shorter duration.

Since some of these children had few or no ailments and no enlarged organs were found, they were not known to be SC haemoglobinopathy carriers.

The anaemia of Hb S homozygous children called for continuous medical attention, which was not the case for most of those with SC haemoglobinopathy. Despite the difference in nature and frequency of ailments between these groups under regular medical supervision, no marked differences in body weight were established.

The older and adult Hb SC carriers. The Hb SC carriers of from 15–25 yrs of age were all healthy with no, or very slight, ailments (these being only of short duration). The haemoglobin concentrations were higher than during childhood. Just as with the older Hb SC carriers these values were found to be higher for the men than for the women. The Hb SC carriers aged between 25 and 50 also had relatively few complaints. In most cases the number of ailments among the older workers with SC haemoglobinopathy did not render them less fit for work. It was, however, found that two of the five male carriers had serious eye troubles which occurred between the ages of 30 and 40. The haemoglobin concentration of the adult Hb SC carriers reached the practically "normal" values for the tropics of 13 and 14 gr%.

The "non-classic" sickle cell-thalassaemia carriers between the ages of 7 and 16 had remarkably few ailments. The family member T 4 had only come to the polyclinic once, and that was because of a headache. His older brother T 3, had been completely free of ailments from 7 to 14, and in three years since he had only had a few brief bouts of pains in the extremities, recurring both spontaneously and after exertion.

Two workers, the brothers Father Q I and Fa. Q II, had had few complaints between the ages of 29 and 36 and 19 and 30 respectively. They were both in excellent physical condition.

On the other hand, the head of the family R had regularly recurring complaints. It is not certain whether all the ailments and spells in hospital of this slightly built man, who had a medical history going back 20 years, were connected with his haemoglobinopathy. It is noticeable that both he and Father Q II had atypical "pneumonia-like" complaints coupled with abnormal findings in chest X-rays.

The haemoglobin concentrations of these workers varied from 11–16 gr% and reached normal values in the adult males.

Workers with the sickle cell trait. Of the workers with the sickle cell trait it was found that:

47	men	aged	between	20	and	40	had required	medical	attention	for	1	to	6 years;
38	„	„	„	25	and	45	„	„	„	„	7	to	10 „
67	„	„	„	28	and	50	„	„	„	„	11	to	14 „
118	„	„	„	32	and	60	„	„	„	„	15	to	18 „

The number of these men who had suffered from haematuria was checked. It was found that seven men with the Hb S trait had been examined in connection with this complaint. In three of them, aged 57, 24 and 32 respectively, the cause of the haematuria (which had only occurred once and had been of short duration) remained unknown after X-ray and cystoscopic examinations.

The medical data for these 270 men did not indicate that other ailments or abnormalities were of regular or markedly frequent occurrence.

Cases with a Hb SF pattern.

The child with "classic" sickle cell-thalassaemia, child U 4, was of normal weight at birth and weighed 8000 gr. and 17.5 kg at 1 and 5 respectively. During the first twelve months he was regularly under treatment for recurrent diarrhoea and retarted growth. When 5 yrs old he was brought to the polyclinic because of pains in the abdomen and back. The spleen was not palpable at the age of 3, but was at 5. The haemoglobin concentration of this child was not recorded.

The child who was heterozygous for both Hb S and persistent Hb F, child V 2, was of normal weight at birth and weigted 10000 gr. when 1 yr old. He had no special ailments until he was 4½, when enlargement of the spleen was established upon him being examined in connection with abdominal pains. During the following six months he was seen three times because of pains in the abdomen and lower extremities. His haemoglobin content was 12 gr%.

The child with a Hb SF pattern, child W 1, was of normal weight at birth and suffered from recurrent diarrhoea until he was 1 year old. At the age of one a splenectomy was carried out owing to hypersplenism of unknown origin. Besides malnutrition and diarrhoea he suffered from serious haemolytic anaemia and thrombocytopenia. Salmonella C_2 and B and also Shigella Boyd were obtained from the stools. Since the operation this anaemic child (Hb 9.5 gr%) has had no specific complaints and has enjoyed reasonable health.

The woman with the Hb SF pattern, mother Y who had four children, was under polyclinical supervision from the age of 27 to 32. The haemoglobin concentration of this woman was never found to be higher than 11.5 gr%. During three pregnancies she had short-lived anaemias of 9.2, 6.5, and 8.5 gr%. This leptosome woman only had asthenic complaints. Her spleen was never found to be palpable.

TABLE 27 — *The Hb S homozygotes.*

Hb S-homozygotes		Origin	Age during control (years)	Duration of control (years)	Polyclinic	Pain crises				Anaemic crises
						Hospital	Musculo-skeletal	Abdominal	Chest	
No.	Sex.									
1	m	Curaçao	0-1	1	2	—	2	—	—	—
2	f	Curaçao	0-1	1	3	2	—	—	—	—
3	m	Curaçao	0-2	2	8	4	3	—	1	1
4	m	Curaçao	0-3	3	2	—	—	—	—	—
5	m	Curaçao	0-3.5	3.5	6	3	4	—	1	—
6	m	Grenada	0-3.5	3.5	15	9	10	1	2	—
7	f	Curaçao	0-5	5	22	13	10	9	—	—
8	m	Curaçao	0-5	5	14	7	4	—	5	1
9	m	Curaçao	0-5	5	16	15	3	4	—	2
9	m	Curaçao	5-10	5	6	3	—	6	—	—
10	f	Curaçao	5-10	5	9	3	1	4	3	—
11	f	Surinam	5-10	5	9	2	4	1	—	1
12	m	Curaçao	6-10	4	6	4	—	—	2	—
13	m	Curaçao	7-10	3	9	4	1	3	4	1
10	f	Curaçao	10-15	5	14	7	6	9	1	—
11	f	Surinam	10-15	5	3	1	3	1	—	—
12	m	Curaçao	10-15	5	—	—	—	—	—	—
13	m	Curaçao	10-15	5	13	7	—	6	6	—
14	f	Curaçao	11-18	7	3	—	—	—	—	—

General complaints	Hb range (gr %)	Spleen palp. up to (years)	Liver	Weight	Particulars
in	7-9	?	?	one year 9000 gr.	polydactylitis.
ver arrhoea	6-9	1	+	one year: 8900 gr.	† cause: crisis in sickle cell anaemia + pyelonephritis.
alaise arrhoea ver	5-10	—	—	two years: 9000 gr.	nutr. deficiency.
petite	8	3	+	three years: 10.500 gr.	at age two: Hb F 26.5%; at age three: Hb F 18.5%.
alaise gh fever arrhoea	8-10	3.5	+	three years: 13.500 gr.	† cause: crisis in sickle cell anaemia.
alaise ver, anaemia	5-9	3.5	+	three years: 16.000 gr.	
alaise ver, anaemia	5-10	5	+	five years: 14.500 gr.	
alaise ver, cough, spnoea	6-9	—	+	five years: 16.000 gr.	
alaise ver, anaemia	5-8	2	+	five years: 16.500 gr.	
in	5.5-8.5	—	+	ten years: 27.000 gr.	
ver aemia	5.5-9	—	+	ten years: 23.000 gr.	
aemia	7.5-9.5	8	+	ten years: 29.500 gr.	
ver spnoea	6-9.5	—	+	ten years: 23.000 gr.	
ver spnoea	4.5-9	+	+	ten years: 26.000 gr.	
in ver	6-8.5	—	—	fifteen years: 38.000 gr.	
in	8-10	—	+	fifteen years: 44.500 gr.	
—	9.5	—	—	fifteen years: 41.000 gr.	
in	6-7.5	11	+	fifteen years: 30.000 gr.	
ad-ache aemia	8-10	—	—	sixteen years: 46.000 gr.	at age 21 in "good" condition, according to parents.

TABLE 28 — *The young Hb SC carriers.*

Hb SC-carriers		Origin	Age during control (years)	Duration of control (years)	Polyclinic	Hospital	Pain crises			Anaemic crises
No.	Sex.						Musculo-skeletal	Abdominal	Chest	
1	f	Curaçao	0-1	1	—	—	—	—	—	—
2	m	Curaçao	0-1.5	1.5	1	1	—	—	—	—
3	m	Curaçao	0-1.5	1.5	1	1	—	—	—	—
4	f	Curaçao	0-1.5	1.5	—	—	—	—	—	—
5	m	Curaçao	0-2	2	4	2	—	—	—	—
6	f	Curaçao	0-2	2	—	—	—	—	—	—
7	f	Curaçao	0-2.5	2.5	2	1	—	—	—	—
8	m	Curaçao	0-3.5	3.5	6	3	6	—	—	—
9	m	Curaçao	0-4	4	2	1	—	—	—	—
10	m	Curaçao	0-5	5	—	—	—	—	—	—
11	m	Curaçao	0-5	5	9	1	5	3	1	—
12	f	Curaçao	0-5	5	1	1	—	1	—	—
13	f	Curaçao	0-5	5	2	1	—	1	—	—
14	f	Curaçao	0-5	5	2	1	2	1	—	—
13	f	Curaçao	5-9	4	1	—	—	1 ?	—	—
14	f	Curaçao	5-9	4	1	1	—	—	—	—
15	f	Curaçao	5-9	4	2	1	1	1	—	—
16	f	Curaçao	5-10	5	1	—	—	—	1 ?	—
17	f	Curaçao	5-10	5	1	—	1	—	—	—
18	f	Curaçao	5-10	5	1	—	—	1 ?	—	—
19	m	Curaçao	5-10	5	1	1	1	—	—	—
20	m	Curaçao	5-10	5	3	1	2	—	1	—
21	f	Curaçao	10-13	3	3	2	1	2	—	1
20	m	Curaçao	10-14	4	6	—	6	—	—	—
22	m	Curacao	10-15	5	1	1	1	—	—	—
23	m	Curaçao	10-15	5	—	—	—	—	—	—

General complaints	Hb range (gr %)	Spleen palp. up to (years)	Liver	Weight		Particulars
—	10	—	—	one year:	9.000 gr.	
icterus	7-11.5	1	+	one year:	12.000 gr.	
generalized edema	10	—	—	one year:	8.000 gr.	
—	9.5	—	—	one year:	8.000 gr.	
convulsions+ fever	8-12	2	—	two years:	11.000 gr.	
—	—	—	—	two years:	12.000 gr.	
nutricional deficiency	11	—	—	two years:	8.800 gr.	
pain in extr.	9-11	—	—	three years:	13.000 gr.	polydactylitis
icterus edema of feet	9-12	4	+	four years:	15.000 gr.	
—	9-11	?	?	five years:	17.000 gr.	
pain	8-10	5	+	five years:	17.500 gr.	
icterus	10-12	—	+	five years:	15.000 gr.	
dyspnoea	9.5	—	—	five years:	16.000 gr.	asthma
pain fever	9-12	4	+	five years:	15.000 gr.	
pain	9.5	—	—			
haematuria	9-10	—	+	nine years:	24.000 gr.	
swelling of leg	10-12	9	+	nine years:	24.000 gr.	no proof of osteomyelitis
pain in chest	—	?	?	ten years:	28.000 gr.	
—	11	—	+	ten years:	23.000 gr.	
pain left side	10-11	?	?	ten years:	30.000 gr.	
swollen upperarm	10	10	—	ten years:	?	
icterus pain	9-11	—	—	ten years:	20.000 gr.	
excessive pain, anaemia	7-12	10	+	ten years:	24.500 gr.	at age 10 splenectomy
pain	9-12	—	—	fourteen y.:	28.000 gr.	
pain in swollen leg	10.5	15	+	fifteen y.:	31.500 gr.	no proof of osteomyelitis
—	10-12	15	+	ten years:	22.000 gr.	

TABLE 29 — *The older Hb SC carriers.*

Hb SC-carriers No.	Sex.		Age during control (years)	Duration of control (years)	Polyclinic	Hospital
22	m	Curaçao	15-18	3	—	—
23	m	Curaçao	15-20	5	4	1
24	m	Curaçao	19-24	5	4	1
25	f	Curaçao	20-22	2	1	1
26	f	St. Vincent	20-25	5	—	—
27	m	Cucaçao	20-25	5	—	—
28	m	Curaçao	22-25	3	—	—
26	f	St. Vincent	25-34	9	1	—
27	m	Curaçao	25-35	10	4	1
28	m	Curaçao	25-40	15	—	—
29	m	Curaçao	26-43	17	25	8
30	m	Curaçao	29-36	7	4	1
31	f	Curaçao	32-42	10	3	2
32	m	St. Maarten	33-48	15	6	4

Pain crises			Anaemic crises	General complaints	Hb range (gr %)	Particulars
Musculo-skeletal	Abdominal	Chest				
—	—	—	—	—	13	
4	—	1	—	pain	11-12	
4	1	—	—	pain	14	
1	—	—	—	pain	8-13	anaemia in pregnancy
—	—	—	—	—	9-10	
—	—	—	—	—	13	
—	—	—	—	—	13	
—	1	—	—	pain	9-10	
4	—	—	—	pain	12-14	recurrent proctitis
—	—	—	—	—	12-14	
12	2	9	—	pain bad eyesight	12-14.5	ablatio retinae
—	4	1	—	pain	14	
2	—	2	—	asthenia	10-11	anaemia in pregnancy † cause: unknown (died at home)
4	—	—	—	pain eye complaints	11-13	retinitis proliferans + haemorrhage

TABLE 30 — *The "non-classic" sickle cell-thalassaemia carriers.*

"non-classic" sickle cell-thalassaemia carriers		Origin	Age during control (years)	Duration of control in years	Polyclinic	Hospital	Pain crises			Anaemic crises
Fam.	Sex.						Musculo-skeletal	Abdominal	Chest	
T 3	m	Curaçao	7-14 14-17	10	6	—	6	—	—	—
T 4	m	Curaçao	9-16	7	1	—	—	—	—	—
S 4	m	Curaçao	11-16	5	2	1	1	1	—	—
S 3	f	Curaçao	12-18	6	2	—	1	—	1	—
Fa Q II	m	Curaçao	19-30	11	8	1	7	—	1	—
Fa R	m	Surinam	20-43	23	9	6	4	3	2	—
Fa Q I	m	Curaçao	29-36	7	2	1	2	—	—	—

General complaints	Hb range (gr %)	Spleen palp. up to (years)	Liver		Weight	Particulars
						no complaints at all.
pain	11-12	—	—	17 years:	63.5 kg.	pain attacks of short duration in extremities, spontaneous and after exertion.
headache	12	—	—			one visit in polyclinic.
pain	12.5-13	—	+	16 years:	45 kg.	generalized shifting pains. Hospitalisation for observation appendicitis; Spleen just palpable. Diagnosis not confirmed.
pain, often headache	11-13	—	—	18 years:	70.5 kg.	
pain	12.5-16	—	—	30 years:	63 kg.	pain attacks of short duration. Hospitalisation: Spleen just palpable. Liver function tests slightly abnormal. No definite diagnosis (virus pneumonia?)
recurrent severe backache	10-14.5	—	—	36 years:	54 kg.	Hospitalisations: 1) extreme backache. 2) atypical pneumonia, no fever, spleen n.p. Chest X-ray: "mottling". 3) atypical pneumonia; spleen n.p. Chest X-ray: "mottling". 4) gravel in ureter? 5) sickle cell crisis. Abdominal pain. Hb 12 gr %. 6) "non-classic" sickle cell-thalassaemia; extreme backache. Hb 13.5 gr %.
pain shoulder and arm	14-15	—	—	38 years:	88 kg.	Hospitalisation: renal calculus suspected, not confirmed.

CHAPTER VIII

DISCUSSION AND CONCLUSIONS

This investigation revealed the presence of haemoglobin S in an average of 6.6% of the Curaçao workers and 5.9% of the married Curaçao women. 6.3% of the men and 5.5% of the women, aged 20–59, were found to be carriers of the single Hb S trait.

The last-mentioned percentage was determined partly from a relatively small number of women aged 50–59 who were not examined for sickle cells during a periodical medical check-up.

The single Hb S trait was found to be present in 5.0% of the women in the 20–49 age group examined during pregnancy checks.

These results correspond completely with the percentage of trait carriers established previously among Curaçao women.

The percentage of male carriers of the single Hb S trait determined by Van der Sar for this island (8.7%), however, differs from the above value for Curaçao workers (6.3%).

This could be due to a difference in composition of the material, as children were also examined in the earlier investigation and a higher percentage of Hb S trait carriers was accordingly observed. As no break-down was made into age groups for the men on one hand and the women on the other, this cannot be gone into further.

It is noteworthy that on the present occasion the lowest percentage of Hb S and Hb S trait carriers in both men and women was found in the 30–39 age group.

In contrast to the previous investigations by Jonxis and Van der Sar, no homozygotes for the Hb S gene were found among the adults examined.

An indication that persons with sickle cell anaemia could become adults was obtained during the examination of the family members of the only worker who died showing sickle cell anaemia symptoms before the multi-family research was begun. His wife had normal haemoglobin and the four children the Hb S trait. The Hb F and A_2 values together with the blood pictures were normal for them all. Whilst the evidence is not conclusive it does appear probable that this father, who died at the age of 36, was homozygous for the Hb S gene.

The fact that no adults with sickle cell anaemia were found among the workers is either due to selection during the pre-employment medical examination or else to only a very small number of these persons reaching adult ages. The latter could also explain why no Hb S homozygotes were found among the Curaçao women. This could, however, also be the result

of the reduced marriage prospects for women with sickle cell anaemia even if they reach marriageable age.

In all cases where the presence of an excess of haemoglobin S was established in adults during the investigation, this was not (and in one case not solely) the result of homozygosity for the Hb S gene, but of double heterozygosity both for the Hb S gene and for another haemoglobin gene. Although the electrophoretic and chromatographic examinations led one to suspect this in many cases, definite confirmation was obtained from the family investigation.

This makes it highly probable that at least a certain number of persons previously recorded as adult sickle cell anaemia cases were not homozygous for the Hb S gene.

The multi-family research showed that the presence of an excess of Hb S in the children was in most cases due to homozygosity for the Hb S gene arising from both parents having the Hb S trait.

In a certain number of cases this was due to interaction of the Hb S gene obtained from one of the parents with a different haemoglobin gene derived from the other. Only in the case of one child (child W 1) was it impossible to explain the genetic background of a high amount of Hb S.

These findings confirm Neel's heterozygous-homozygous hypothesis.

The haemoglobin distribution among the children also corresponds with what was to be expected in accordance with this hypothesis.

In the families examined after a morbid syndrome had been found in one of the children (Dr. Winkel series), the clinical approach was reflected in a relatively higher number of children with sickle cell anaemia or Hb SC disease. The ratio between the number of male and female children in the families examined was found to be about 1 : 1. The ratio between the total number of boys and girls from the families where one parent had the Hb S trait and the other normal haemoglobin is here an exception. It is not clear what significance must be attached to this.

As a result of the extension of the investigation in families where parents or children had a Hb SAF or Hb SF pattern, it was possible to prove that two different thalassaemia's occur on Curaçao. The persons in whom a Hb SAF pattern was established originated from Curaçao, Surinam and St. Vincent.

With the exception of the child from the last-mentioned island, in whom the thalassaemia type could not be determined, all the others were found to be heterozygous both for the Hb S gene and for a thalassaemia gene indicated as "non-classic".

Heterozygosity for this gene manifested itself by increased Hb A_2 either with or without increased foetal haemoglobin with minute morphological abnormalities of the erythrocytes which were very difficult, and sometimes impossible, to establish.

It is highly probable that this "non-classic" thalassaemia on Curaçao is the same as that which caused increased Hb A_2 and Hb F but no morphological abnormalities of the erythrocytes in a West Indian negroid student in Jamaica, as observed by Went and MacIver.[86]

It appears likely that this thalassaemia occurs specially in individuals of negro descent.

The fact that more classic thalassaemia was found in Jamaica than in this material may be due to a different composition of the population on that island, where classic thalassaemia was also found in Chinese. It is possible that the nature of the material examined results in the "non-classic" thalassaemia being encountered more frequently than the classic in Curaçao.

The fact that thalassaemia major is never met with on this island with its many children does, however, lead one to presume that classic thalassaemia does indeed occur less frequently on Curaçao, or at least that the "thalassaemia situation" is different there.

Following family investigations arising from the Hb SF pattern in one of the family members it was also shown that the persistent high foetal haemoglobin anomaly occurs. The presence of this anomaly was apparent from the presence of increased Hb F in the relevant order of magnitude and from the low normal Hb A_2 values together with the increased osmotic resistance.

The absence of heterozygotes for both the Hb S gene and the persistent high Hb F anomaly among the adults examined could be owing to the infrequent occurrence of the persistent high Hb F anomaly among the negroid population of Curaçao. However, at the time the family investigation was carried out the presence of increased foetal haemoglobin together with normal adult haemoglobin was repeatedly observed during routine electrophoretic examinations. Likewise three persons whose haemoglobin was examined in connection with enlargement of the spleen were found to be heterozygous for the Hb C gene and the persistent high Hb F anomaly.

This leads one to suppose that this anomaly is not after all so scarce among Curaçao's negroid population. If this is in fact the case the lack of adult Hb S-persistent high Hb F carriers in this series of tests would be owing to the fact that erythrocytes cannot easily sickle in the presence of this haemoglobin combination. This is not, however, borne out by the results

for child V 2, whose blood quickly showed the sickle cell phenomenon.

It would appear from the findings in the family Y that a Hb SF pattern can be due to another genetic basis than those already known (homozygosity for the Hb S gene; double heterozygosity for the Hb S gene and thalassaemia; heterozygosity for both Hb S and persistent high Hb F). In the children belonging to the mother with the Hb SF pattern the ratio between haemoglobin A and S was the same as that of the ordinary sickle cell trait. In three of the children an increase of the Hb F and the number of target cells was established.

These findings are similar to those found by Aksoy among the Eti-Turks and which were described by him as expressions of sickle cell-thalassaemia.[2] Whether the findings for these children and the mother from St. Eustatius are due to the presence of thalassaemia can neither be proved nor disproved*.

It was possible to obtain a general idea of the fitness of the Hb S carriers, the nature of whose haemoglobin had been established by electrophoretic examination. This indicated that the young Hb S homozygotes were the most handicapped, even if the individual syndromes as such are not taken into account. There are indications that a regular control contributes towards some of them being able under more favourable conditions to reach an age at which their lot usually improves.

The haemoglobinopathy of the young Hb SC carriers did not appear to affect them unduly in this respect and had little influence on their fitness.

Whilst the older Hb SC carriers were in most cases in good general condition, the number of complaints varied from individual to individual. It would appear desirable when allocating employment to SC haemoglobinopathy carriers to bear in mind that serious chronic eye troubles may occur when the carriers are still relatively young.

As far as could be deduced from the first observations, "non-classic" sickle cell-thalassaemia does not constitute a serious handicap for the carriers.

As regards the adult male carriers of the single Hb S trait, is was not found that certain ailments which could have been connected with their haemoglobin type were of frequent occurrence. If the incidental occurrence of unexplained haematuria was due to the presence of the sickle cell trait, it does not even then affect the fitness of the men with the sickle cell trait under the tropical conditions on Curaçao.

* It is fascinating to consider that at the time St. Eustatius was called "The Golden Rock" there were Turks living on the island (J. and D. Keur in "Windward Children". a study on the human ecology of the three Dutch windward islands in the Caribbean).

SUMMARY

This study describes the methods followed and the results obtained from multi-family research on the mode of occurrence of the abnormal haemoglobin S among negroids on the island of Curaçao. Other haemoglobins are also dealt with in so far as they were encountered in combination with haemoglobin S.

The investigation was carried out on this island between 1959 and 1962, and covered some 200 families belonging to workers of Shell Curaçao N.V. and originating from Curaçao, the Leeward and Windward Islands, the small British islands in the Caribbean and Surinam.

Paper electrophoresis was utilized for the haemoglobin investigation, which was based on the positive sickle cell test results obtained during periodical medical examinations of men and women as carried out by the medical service of the above company.

Following the findings for parents or children the investigation was extended in a number of families. In these cases beside chromatography and alkali denaturation together with haematological examinations, Tris buffer electrophoresis was applied for semi-quantitative determination of the Hb A_2 fraction. This latter method, developed by Dr. E. D. A. Sindram, is described.

The results of this study relate to 1) the occurrence of haemoglobin S among workers and married women on Curaçao, 2) the mode of occurrence and distribution of haemoglobin S and other haemoglobins among the members of the sibships, 3) the occurrence of two thalassaemia types together with persistent high foetal haemoglobin on the island, and 4) the influence of haemoglobin S on the fitness of the carriers as shown by frequency data for ailments and diseases.

RESUMEN

La presente tesis describe los métodos seguidos y los resultados obtenidos en una investigación en escala multifamiliar para determinar la forma en que se presenta la hemoglobina S anormal entre los negroides en la isla de Curazao. Este estudio se refiere también a otras hemoglobinas, por cuanto éstas han sido encontradas en combinación con la hemoglobina S.

La investigación se llevó a cabo exclusivamente en la citada isla, en el período comprendido entre 1959 y 1962, extendiéndose a unas doscientas familias de obreros de la Shell Curaçao N.V., provenientes de Curazao, de las otras islas de Sotavento, de las islas de Barlovento, de las pequeñas islas británicas en el Caribe y del Surinam.

La investigación de hemoglobina que se realizó con el método de electroforesis con papel, se basaba en los resultados positivos de ensayos de células falcadas, obtenidos de determinados hombres y mujeres durante los controles periódicos efectuados por el servicio médico de dicha compañía.

Con motivo de los resultados obtenidos de padres o hijos, se procedió, en un número de familias, a una investigación más profunda, aplicándose, aparte de cromatografía, desnaturalización por álcali y exámenes hematológicos, la electroforesis denominada Tris buffer para la determinación semicuantitativa de la fracción Hb A_2. Este método, inventado por el Dr. E. D. A. Sindram, está descrito en esta tesis.

Los resultados de este estudio se refieren a 1) la presencia de hemoglobina S entre empleados y mujeres casadas de Curazao; 2) la forma en que se presenta, y la distribución de la hemoglobina S y otras hemoglobinas entre los descendientes en primer término y en linea horizontal; 3) la presencia de dos tipos de talasemia, junto con un contenido constantemente elevado de hemoglobina fetal en la isla, y 4) la influencia de la hemoglobina S en la aptitud física de los portadores, tal como la manifiestan los datos relativos a la frecuencia de afecciones y enfermedades.

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